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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHOD AND DEVICE FOR MANIPULATING LIQUIDS IN MICRO-FLUIDIC SYSTEMS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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Method and device for manipulating small volumes of multiple liquid components in micro-fluidic systems (reactors, processors) with prefabricated non-uniform capillarity.

V.B. Gorfinkel, E.A. Kabotyanski,

Abstract:

We describe a design and construction of a micro-fluidic system (Assembly or Chip / Plate) comprising micro-fluidic reactors, channels, chambers, openings, etc., with specially designed and fabricated areas of enhanced/reduced capillarity (flow guides), and operation of the said system, which enables bubble-less loading, dispensing, mixing, sequencing, etc., of small volumes of multiple liquid components into the said system for the use of the said system in various applications including, but not limited to, real-time PCR, capillary electrophoresis, processing of DNA samples for DNA sequencing, and a combination of the above mentioned applications.

Vocabulary

Air encapsulation
Array
Capillarity
Capillary
Capillary Electrophoresis (CE)
Microfabrication
Microfluidics
Polymerize chain reaction (PCR)
DNA sequencing

Prior Art

Development of versatile microsystems for DNA amplification with polymerase chain reaction (PCR) and DNA analysis with capillary electrophoresis (CE) has become an important direction in current biotechnology since such devices were demonstrated in 1993 (Northrup et al.). These instruments are manufactured as sandwich chips or wafers from silicon, glass, or plastic substrates using scalable microfabrication techniques originated in semiconductor industry. Microfabricated devices allow overcoming such limitations of conventional PCR as long assay times, large, expensive volumes of reaction components, etc. Examples of recent designs include fully integrated PCR-CE microfluidics

devices (Lagally et al., 2000-2003), 384-lane CE array (Emrich et al., 2002) (additional references to the field could be found in the cited articles). Further progress in design development and application of on-chip DNA analysis technology is hampered, however, by difficulties derived from inherent properties of microfluidics and from handling of microfluidic devices. The problems occur when connecting to macro-devices, precise loading of very small samples, mixing of several components, because of cross-contamination, etc. One of the principal problems is the air encapsulation, or forming of air bubbles clogging microfluidic channels and disturbing loading and mixing of samples. This problem forces designers to employ various additional precautions and devices (e.g., vacuuming, pumping, microfluidic valves and vents) that in turn make the technology less versatile and more expensive.

Another part of the prior art is using capillaries with filaments for bubble-less filling of micropipettes, microelectrodes, etc. (Brown and Flaming, 2001) (see Fig.1). The latter formations, however, are essentially tubes open from both ends, and their function is to provide electrical conductivity between two ends. They are not supposed to be closed from one end (tip), and they do not function as test-tube or reactor. Although one can fill the micropipettes, microelectrodes, etc., with more than one liquid component without babbles, these components fill the capillary sequentially and do not mix well.

Claim

We are proposing a novel method and device (article) for practicing the method for manipulating small volumes of multiple liquid components in micro-fluidic systems with prefabricated non-uniform capillarity.

Said device is a micro-fluidic system comprising micro-fluidic reactors, channels, chambers, openings, etc. (Assembly or Chip / Plate) with specially designed and constructed sectors of enhanced/reduced capillarity. These sectors are specially pre-fabricated structures/formations/profiles that provide enhanced (or reduced) capillarity (super-capillary flow guides/ducts, or anti-capillary flow guides/ducts). Certain procedures and sequences of manipulating said multiple liquid components are used, such as a specific order of filling /loading the liquid components (samples) into channels/chambers of said system; touching only the flow guide, not the rest of the said channel/chamber, with pipette's/dispenser's tip during the loading procedure, which is critical for preventing air encapsulation. The said constructive (i.e., flow guides) and operational solutions enable bubble-less dispensing, loading, mixing, separating, etc., of small volumes of liquids within reactor channels/chambers of the said system (Fig. 2a).

Said method and device (system) can be used for various applications including, but not limited to, carrying out chemical reactions with certain protocol, PCR, real-time PCR, capillary electrophoresis, processing of DNA samples for DNA sequencing, combinations of the above applications.

Said method and device comprise:

A multitude of microfluidic channels, chambers, capillaries, or any other cavities which have pointed non-uniformities (increase or decrease) in capillarity across their saggital perimeter (Fig. 2b). Said non-uniformities comprise narrow sectors bearing enhanced (or reduced) capillarity in /on the walls of

said cavities as compared to the rest of the wall. Said features comprise structures/formations/profiles of enhanced (or reduced) capillarity that are positioned within and along the length of said cavities (microfluidic capillary chambers/channels) (Fig.2b,c; Fig.3). Said features are termed flow guides/ducts (var.: sub-capillaries, co-capillaries, super-capillaries; or anti-capillaries, anti-guides). Said flow guides form uninterrupted flows of said liquids of interest, prevent formation of air bubbles that can clog microfluidic channels during their filling with said liquids, and enable bubble-less dispensing, loading, mixing, separating, etc. of small volumes of said multiple liquid components within said cavities of said system. Multiple liquid components of interest can be loaded into said cavities/channels through individual or common loading ports (Fig.2c).

Flow guide embodiments:

- There are two major types of flow guides.
  - One type is represented by areas of enhanced capillarity that are prefabricated by changing profile geometry of microfluidic chamber(s) (Figs 2b, 3a-j).
  - Another type is surface flow guides (Fig. 3K,L) that can be made by deposition of hydrophobic materials on hydrophilic substrates (diamond-like carbon on glass substrate, carbon nano-tubes on glass or on metal strips).
- there can be one to many per microfluidic chamber/channel;
- can be positioned parallel to each other or join in V-like, Y-like, star-like, or any other manner;
- can be of equal or varying length; can span the whole length of the microfluidic chamber, or can be shorter than the latter at one or both ends (Fig. 2c);
  - a flow guide that spans the length of the microfluidic chamber and contacts with the chamber's wall at one of the ends will serve as a duct delivering fluid first to that end of the chamber; when fluid contacts the wall of the microfluidic chamber, it starts filling the chamber from that end due to chamber's own capillary properties; this will provide controlled bubble-free filling of the microfluidic chamber/channel with a large volume liquid component (Fig. 2c);
  - a flow guide that does not contact the microfluidic chambers' wall at the ends can be loaded with a small volume of liquid, which said flow guide will hold on itself due to its own capillarity; this will provide gradual and distributed mixing of said small volume liquid component as the large volume liquid component fills the microfluidic chamber (Fig. 2c);
- In order to prevent spill-over and un-intended mixing of different liquid components during their loading into microfluidic chambers, the flow guides will be separated by anti-guides, which will be formed by hydrophobic areas or strips (Fig. 3K, L). They can be fabricated by any appropriate technology, e.g., by deposition of special hydrophobic films, coatings, micro- or nano-particles on

flat, protruding or etched area of the substrate. A particular separation design can be represented by a super-capillarity grove/notch surrounded by a hydrophobic border (Fig. 3 i)

- flow guide types (Fig.3): etched or carved cracks, slits, notches, groves, flutes (Fig. 3,a,c,f, and i); attached, soldered or etched (micro-) filaments, tubes/capillaries, fibers, ridges/blades/edges (Fig. 3b,d, and e); regions of deposited/plated/coated material(s) with extra high capillarity, such as micro- or nanoparticles (Fig. 3g,h, and i); features formed by protruding or invaginating edges of sandwich layers (Fig. 3j); regions of processed or altered surfaces of said cavities (e.g., a strip on the channel wall created by laser blasting, mechanical surface processing, such as sanding, scratching, etc.); as well as combinations there of (Fig. 3h and i);
  - micro- or nanoparticles can be spheres/beads, tubes, tips, edges, micro-cracks, grinded powders, etc., made of glass, carbon, oxidized metal, and many other materials. Choice of material depends on the purpose. If micro- and nano-particles are used for flow guiding, material must be hydrophilic (glass, oxidized metal). For surface flow guides hydrophobic (carbon) micro-, nano-particles can be used.
  - sandwiches can be made of glass frames (Fig. 3j), or layers of Si substrate, glass frame, and glass cover slip, assembled and held together either by gluing, ultrasonic soldering (bonding), lapping, polishing.

- flow guide can have various profile geometry in cross-section (round, oval, triangular, rectangular, trapezoid, and polygon shape) and along its length (cylinder, cone, prism, parallelepiped, polyhedron, pyramid, etc.) (Fig. 2b, 3); cross-section profile can vary/change along its length in shape or size (Fig. 4 and 2c);
- flow guide can be straight, curved (sine-wave, spiral, etc.), with appendices, crisscrossing (net-like or comb-like) (Fig. 2c);
- flow guide can be a combination of the above indicated types, shapes, geometry, and sizes (e.g., Fig. 3, h, i and j);
- capillarity action can vary along or across a flow guide; the degree of capillarity can be controlled by controlling say a shape of the flow guide (e.g., angle and depth of the Small volume (sample) holding flow guide on Fig. 4), material it is made of.
- amount of said liquid component that flows along or held at a flow guide can vary along its length by varying flow guide volume or its cross-section profile; and can differ between flow guides;
- flow guides in micro chambers/channels can be formed by multiple technologies, including but not limited to, etching, engraving, carving, press-formed (mold pressing), extrusion through a draw plate, laser burst, laser cutting, film deposition, soldering/attaching, forging/drawing, etc.
- Surface flow guides will be created on chip by forming hydrophobic (or reduced capillarity) areas/zones/strips and combining them with areas of enhanced or normal capillarity. Particularly,

said areas of reduced capillarity may surround said areas of enhanced capillarity, and thus form surface flow guides (Fig.3, K, L).

Depending on their design, said surface flow guides can perform sample holding function as well as chamber filling function (Fig. 3.L). In order to ensure mixing of liquid components in the chamber, flow guides can have openings in their reduced capillarity (hydrophobic) areas (Fig 3L).

Said surface flow guides can be formed by any appropriate fabrication technology. For example said areas of reduced/enhanced capillarity can be fabricated by deposition of special films, coatings, micro- or nano-particles (nano-spheres, nano-tubes, nano-clasters, etc.) on flat, protruding or etched area of the substrate (Fig.3L)

Said surface flow guides can have special designs enabling them to perform the same functions as flow guide types describe above.

Microfluidic chamber(s)/channels include but are not limited to:

- single or multi-chamber/channel arrays;
- assembled from single or many capillary tubes (Fig. 5a);
- can be manufactured by:
  - scalable microfabrication with etching, on glass, or silicon, or plastic, or combined chip (plate) (Fig. 5b);
  - carving, engraving, sand blasting, laser burst, laser cutting on a glass/silicon/plastic;
  - assembled from prefabricated glass frames (Fig. 3j);
  - extrusion through a draw plate, compaction/mold pressing;
  - forged/pulled from a pre-fabricated glass ingots (Fig. 6,7);
  - or a combination of the said and other methods;
- may have hydrophobic coating of any shape, geometry, length, etc., at any place inside or outside;
- may vary in size and volume, profile geometry at cross-section (round, oval, triangular, rectangular, trapezoid, and polygon shape) and along its length (cylinder, cone, prism, parallelepiped, polyhedron, pyramid, etc.) (Fig. 2b); cross-section profile can vary/change along its length in shape or size;
- may have pre-fabricated layers or strips of deposited/plated/coated material(s) inside or outside (Fig. 14, 15)
  - metal of various shape, thickness, size and position to function as electrodes, heaters, mirrors, sensors, etc.;
  - semiconductors – as thermal pumps (heater/cooler), sensors, etc.

- may have pre-fabricated optics – lenses, filters, dichroic mirrors, prisms, optical waveguides (Fig. 8);
- may have pre-fabricated mechanical parts (stirrers, etc.)
- monolith, two or multi-layer sandwich, assembly or a combination of above.

Using the inventive device and method for PCR or PCR/CE assays

One preferred embodiment of said device (further called PCR or PCR/CE processor or Processor or Reactor) and method is an assembly of capillaries (multi capillary array) or microfabricated chip (glass, silicon, plastic, or a combination there of) used as a vehicle for multi-channel PCR, real-time PCR, PCR-CE, and other applications that involve dispensing, loading, mixing, thermal cycling, separating, etc., of small (microliter, sub-microliter and/or nanoliter) volumes of multiple liquid components.

The PCR or PCR/CE processor may comprise only PCR chambers (Fig. 8), or may be integrated with DNA analysis (CE) sub-unit/device/capillary (Fig. 9).

- Said Processor can be assembled from capillary tubes (Fig. 9) or microfabricated or carved on chip (Fig.10). It can be designed for real time PCR (Fig.10a) and used in horizontal, vertical or inclined position.
- Said Processor can be used for both diagnostics and production purposes.

I. Ports (openings) for loading (filling) microfabricated Processor (Reactor) with liquid components and unloading (sampling) products from the Processor (Reactor).

(Procedures described below are applicable to various microfluidic systems including, but not limited to, PCR and PCR/CE processing)

Processor's channels/chambers/cavities can have one to several specially designed openings - Loading Ports for filling them with different liquids (DNA/RNA samples or reagent solutions/mixes) (Fig. 4, 11, and 16). Additional port(s) can be made to release/vent air out of the Processor, to gain access to stirrers, and to gain access to liquid mixture inside the chamber. Reaction products can be taken from the Processor for further analysis or purification. For this purpose, additional port(s) can be fabricated and additional procedures can be added to the operation protocol.

The loading/unloading ports will be sealed (with PCR oil or using polymer films/layers, or some other known methods) to temporarily block them and/or secure sterile and clean (RNAse free) interior reactor space.

Unloading Port(s) can be prefabricated at the distal (bottom/blind) end of the chamber. After the end of reaction, plastic seal will be removed to open said port(s). A capillary or a needle will be inserted to draw out the PCR product from individual PCR chambers. Said port can be also used for taking a sample into CE capillary. The inlet (tip/end portion) of the latter will be inserted into the port, an appropriate

voltage will be applied between the inlet and another electrode and a sample will enter the inlet of the CE capillary by means of electro kinetic injection.

Unloading can be also performed via loading ports if an additional designated unloading port is undesirable. After the end of reaction cycles, a liquid (e.g., distilled water, buffer, or sequencing reaction mix) will be added into the Processor chamber so that it is filled past the port's opening. Reaction products will be mixed using alternating DC voltage pulses applied between prefabricated electrodes (see above and also Fig.14). Either a sampling or a CE capillary will be inserted into the port. If needed, the sampling/CE capillary can be tight-fitted into the port. Reaction products will be either drawn out via sampling capillary, or electro kinetically injected into inlet of the CE capillary.

II. Filling of the Processor (Reactor)

Specified sequence of filling steps with different liquid components will prevent cross-contamination between channels AND contamination of stock reagent (e.g., PCR mix). This, in turn, will allow reusable loading tools or procedures for stock reagents / components. (See below and Figs.11a and 16 for detailed loading descriptions and sketches).

If mixing of reaction components, e.g., PCR mix and sample, occurs before their loading, the microfluidic reactor chamber can be filled via one port and one guide. If two or more components must be mixed simultaneously inside the reactor chamber, they can be loaded through the same port and guide in series. In this case, liquids will fill up the chamber in layers, however, and diffusion between these layers may become a negative factor.

The homogeneity of the mixing of reagents in the chamber will be ensured via special design of the flow guides and filling procedure (Fig. 2c, 11a). Two functional types of flow guides can be used. One type is a flow guide that does not contact the microfluidic chamber's wall at the ends and holds a small amount of a liquid at its own walls because of its own capillarity. By varying profile geometry along such a flow guide, one can vary both the total amount of a liquid held there, and lengthwise distribution of the liquid. If a profile of the guide is uniform, the liquid will be distributed evenly alongside the guide. In other instances, guide's profile will expand or narrow in certain points or lengths, thus allowing holding the bulk of the liquid at a desirable region/area of the Processor (Fig.2c, 4, 11a, 12). This type is termed holding flow guide.

Another flow guide type contacts with one of the walls of the Processor's chambers/channels at one of the ends and delivers liquid component to said end of said chamber/channel (Fig.2c, 4, 11a,12). When said liquid component contacts the wall of said chamber, it starts filling the chamber from that end due to chamber's own capillary properties; this will provide controlled bubble-free filling of the microfluidic chamber/channel with a large volume liquid component (Fig.2c, 11). This type is termed large volume flow guide.

Small volume liquid(s) will be loaded first and will be confined within their designated holding flow guide(s) down the length of the reactor chamber (Fig.11a, A). There can be several such holding flow guides when several liquid components are to be mixed. To provide mixing of said liquid components, the

filling procedure will be concluded by loading of a large volume liquid component (e.g., PCR mix) or a solvent via specially designated large volume liquid port and large volume flow guide (Fig.11). The large volume liquid will start filling the reactor capillary chamber from its distant end (Fig.11a,B), and, as it fills, it will mix with said small volume liquid component(s) held at its (their) holding flow guide(s) (Fig.2c, Fig.11a,C).

In case there is another liquid component (or components) which must be added and mixed in later in (e.g., mix for sequencing reaction), it can be loaded via large volume guide. Such a procedure will fill up the chamber in layers, however.

Example 1

Experimental results on bubble-less filling and mixing liquid components in capillary with two flow guider (Fig.11b).

We have fabricated a glass chamber with two flow guides. Holding flow guide was made of thin capillary **(A)**. It did not touch the distal chamber wall and was used for holding a small volume liquid component (SVLC) **(B)**. Another flow guide, which touched the distal chamber wall, was used for bubble-less filling of the chamber with large volume liquid component (LVLC) **(C).** We also observed a mixing of LVLC and SVLC which occurred along the length of the holding flow guide while the LVLC was moving towards the chamber inlet **(D)**.

Better mixing can be obtained using additional procedure(s) and device(s):

1- Adding later component on top of PCR-sample mix, then pumping in and out some air through a special port made at the distal end of the reactor chamber (using either vibration by bass membrane or micro pump). By moving the liquid column along the Processor's chamber, last component will mix in due to the interaction of liquid components with the chamber's walls. During PCR process, this air port will be sealed (e.g., Fig.11.b).

2 – Additional chamber with its own flow guide is prefabricated parallel to the main reactor chamber and separated with the latter by a thin wall, having dimensions enough to line up the later component along the first reactor mixture. This chamber is filled separately. Then the thin separating wall is cracked open by using resonance sound, e.g., ultrasound gun, irradiated from above or below the chip.

3 – Same as 2, but make the thin wall controllable by applying a signal (e.g., voltage to a piezo crystal), so that it vibrates, bends, curves or shrinks, thus letting the liquids to mix.

4 – Same as 2, but the thin wall is porous, and the pores are hydrophobic inside. The first reactor mixture stays confined within the main reactor chamber. After reaction (e.g., PCR), additional chamber is filled. Liquids in both chambers bridge pores, collapse, and start mixing by diffusion. An air puff can be added at the end to push the later component out of its chamber through the pores (for this, envisage filling the additional chamber via tight port).

5 - Adding later component (DNA sequencing mix) on top of PCR-sample mix, then move negatively charged DNA molecules around the chamber with alternating DC current pulses for better mixing.

6 – Prefabricate a stirrer inside the chamber – a filament/straw attached only at the proximal end (near the loading ports). A) Make the core of the stirring straw from electro-controlled material (piezo crystal, bi-metal, etc.) that will bend under voltage or current, with electrodes extended outside the chamber. By applying alternating signals to the electrodes, the straw will stir and mix the liquids. B) Make the core of the stirring straw from magnetic material. Bring vibrating magnet probe close to the straw. The straw will stir and mix the liquids. C) Make the stirring straw from glass/silicon by etching, and make an opening above it. Touch the straw by a vibrating probe through the opening - the straw will stir and mix the liquids. D) Design an opening at the proximal end of the reactor's chamber so that a stirrer straw can be inserted into its cavity.

7– Filling of flow guide channel with multiple liquid components (Fig. 3L).

In case when there are multiple liquid components to be added into a flow guide channel it can be done sequentially by using multiple holding/exchange areas, (Fig. 3L, lower right corner)

8 – Combined filling of capillary assembly with flow guides and pumps (Fig.11.b)

III. Reagent Mixing

It is preferable to well mix reagents before and during reaction(s). One solution is prefabricated stirrers attached inside the chamber (see point 6 above). Another solution will be via the use of nano- or micro-stirrers. They will be made from cut nano- or micro-tubes or bars by a known process. The stirrers will be given a magnetic or an electric dipole and filled/fed into the chamber along with liquid components. Once the chamber is filled, nano- or micro-tubes will be stirred remotely by applying controlled alternating pulses of magnetic or electric field via induction coil or capacitor, correspondingly. The latter can be prefabricated on the chip or be a part of the instrument that handles the chip. The stirring action may continue during all cycles of PCR, which will significantly speed up the process. In addition, since in small volumes surface effects grow much more prominent because of the dramatic increases in surface to volume ratio, continuous stirring will significantly compensate/neutralize negative surface effects caused by non-uniformities in surface tension, profile geometry and diffusion; chemical and electro-static effects on glass surface; etc.

IV. Use of Capillary Reactor as Genomic Multi-array tool for Diagnostics.

In genomics (hybridization arrays, hybridization assays), multiple DNA probes are attached on chips during pre-fabrication. We will attach 600-1000 primers for known gene markers of human/animal cancers to Microfluidic Capillary Chambers. DNA constructs will be attached to inner surfaces of the chambers during their prefabrication. There can be either one probe per chamber, or probes can be attached in stripes, several per chamber, reflected in corresponding coding of each kind of chip/assembly. Then the chip or assembly will be filled with a mixture of PCR mix and sample from an individual patient,

real-time PCR will be performed, and chip is analyzed. Such chip together with PCR instrument will be used as routine on-bench diagnostic tool.

V. Automated and precision loading of liquid components into microfluidic device

Clearly an important factor that limits development of micro-, submicro-, or nano PCR is the step of loading of micro-, sub-micro-, or nanoliter volumes of samples and other components into a capillary chamber via small (10-300 μm diameter) openings/ports. Manual pipetting of sub-microliter volumes lacks precision and repeatability, prone to spill outs, cross-contamination and other human errors. Existing robotic loading stations are not satisfactory. For example, Microlab 4000 by Hamilton Company (Reno, NV) is complex, cumbersome, and expensive, not integrated with PCR machine, and has constructive flaws (e.g., uses washout instead of disposable tips).

Suggested method: Solution loading (and unloading) via flexible capillary tube with disposing of a used portion of the capillary after each step (Fig. 16). The flexible capillary tube is stored coiled (e.g., on a bobbin). Reactor array is moving on a 3D programmable stage along with a sample tray, while loading unit is stationary.

Loading steps (Fig. 16):

1. Stage positions sample tray so that sample well 1 is under sample loading capillary **A.**
2. Capillary **A** is pushed/protruded downward to sample 1 for a measured distance.
3. Stage is lifted up so that sample solution 1 in the sample tray is in contact with loading capillary **A**. The capillary is filled with sample 1 (preferably by capillary force). Precise timing of Pressure control system is used to provide exact volume of the sample in the capillary. (A designated optical device and calibration will be added to precisely control filling of the capillary to desired volume).
4. Stage descends down.
5. Stage moves sideways and positions chip so that PCR/reactor chamber 1 is under the loading unit. Sample loading port of the chamber is exactly under sample loading capillary **A** projecting into the opening; PCR loading capillary **B** projects exactly into opening of PCR mix loading port.
6. Stage ascends, the loading capillary **A** touches floor of PCR/reactor chamber exactly at the sample holding flow guide.
7. Sample is dispensed from the capillary **A** into the holding flow guide by slow air puff from the Pressure control system.
8. PCR mix loading capillary **B** is pushed/protruded downward to touch the floor of PCR/reactor chamber exactly at PCR mix (large volume) flow guide.

9. Required volume of PCR mix (or a large volume liquid) is slowly dispensed from the capillary **B** into the PCR mix flow guide by precise action of PCR mix delivery control system. The reactor chamber is filled via this flow guide and two components are mixed.
10. Stage with the chip descends.
11. Guillotine **G** cuts used portions of both loading capillaries **A** and **B** into a collector.
12. Stage goes to step 1 to work with next sample well and PCR/reactor chamber.

VI. Real time PCR and sequencing detection systems (Fig.13)

There is a variety of optical systems and photodetectors which can be used for detection of the PCR product and CE separation on the preferred PCE/CE chip. Optical system can have separate illumination for individual PCR chambers and CE channels as well as one or several light sources (lasers, LEDs) which illuminate several chambers (or CE channels) simultaneously.

Design of the optical detection system can be based either on open optics or on fiber optics or comprise both open optics and fiber optics features.

Detection of different channels (and different chambers) can be done in a multiplex mode or simultaneously. In case when detection is carried out simultaneously one can use a multi-pixel photodetectors or single pixel detector. In case of the single pixel detector, illumination of individual PCR chambers and/or individual CE channels must be done by multiple light sources with encoded output power.

Preferred Embodiments

Preferred Embodiment 1. Surface (flat) flow guides created on chip by forming and combining areas of enhanced and reduced capillarity (Fig.3K, L).

Preferred Embodiment 2. Monolith multi-capillary PCR/CE array with varying channel cross section pulled from ingot (Fig.7)

Preferred Embodiment 3. Microfluidic reactor chamber(s) arrayed and fabricated of silicon (Fig.12a,b).

Preferred Embodiment 4. Microfluidic reactor chamber(s) arrayed and fabricated of glass (Fig.12c,d)

Preferred Embodiment 5. PCR-CE glass chip fabricated by combination of mold pressure, etching and laser blasting (Fig.12 e)

Preferred Embodiment 6. Real time PCR and sequencing detection systems (Fig.13)

Preferred Embodiment 7. Placement of CE electrodes on PCR/CE chip (Fig.14)

Preferred Embodiment 8. Thermal cycling means and thermal sensors for PCR/CE chip (Fig.15)

Preferred Embodiment 9. PCR mix and sample loading station (Fig.16)

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FIGURES

Fig.1. Bubble-less filling of a capillary with filament

Fig.2a. Loading liquid component into chamber/channel of the microfluidic chip using pipette or capillary dispenser.

Fig.2c. Micro-channel with loading ports and two flow guides for loading and mixing two liquid components.

Fig.3. Flow guides of different types.

Fig. 3K. Surface (flat) flow guides created on chip by forming and combining areas of enhanced and reduced capillarity.

Fig.3L. Possible configurations of surface (flat) flow guides created on chip by forming and combining areas of enhanced and reduced capillarity.

Fig.4. Loading ports (openings) for filling Microfluidic chamber.

Fig.5. Microfluidic chambers assembled from many capillary tubes.

Fig.5a. Microfluidic chambers manufactured by scalable microfabrication with etching on glass, or silicon, or plastic, or combined chip (plate).

Fig.6. Fabrication principle of multi-capillary PCR/CE array.

Fig.7a. Monolith milti-capillary PCR/CE array with varying channel cross section pulled from ingot.

Fig.7b. One channel of the monolith milti-capillary PCR/CE array

Fig.8. Pre-fabricated optics on/at microfluidic chamber.

Fig.9. PCR/CE Reactor assembled from arrayed capillaries and PCR tubes with flow guides.

Fig.10. Integrated PCR/CE array fabricated on chip.

Fig.10a. Integrated PCR/CE array fabricated on chip.

Fig.11.a. Filling microfluidic chamber with two liquids via two flow guides.

Fig.11.b. Loading two liquid components into a glass chamber with two flow-guides

Fig.11.c Bubble –less loading of two liquid components into a glass chamber with one flow-guide

Fig.11. c Combined flow guide and pumping Reactor filling.

Fig.12a. Preferred embodiment (PCR/CE silicon chip).

Fig.12.b. Preferred embodiment (chip cover, Si).

Fig.12.c. Preferred embodiment for PCR/CE chip fabricated on glass.

Fig.12.d. Drawing for PCR/CE chip fabricated on glass.

Fig.13. Real time PCR and sequencing detection systems.

Fig.14. Placement of CE electrodes on PCR/CE chip.

Fig.15. Thermal cycling means and thermal sensors for PCR/CE chip.

Fig.16. PCR mix and sample loading station.

Enablement Questions

1. Touching the flow guide without touching anything else. Is there a known apparatus that would work for me here to which you can refer me?

Touching the flow guide without touching anything else might be executed manually with the help of a pipetter with very thin tip.

One can also use a robot with very precision positioning system. Of existing robots, Microlab 4000 by Hamilton Company (Reno, NV), which we referred to in the NTD (p. 9) may work for 1mm channel width. In general, we doubt that existing pipetting machines will work with all possible designs we proposed. That is why we had developed and presented a separate design for Automated and precise loading (and unloading) of liquid components into microfluidic device via flexible capillary tubes (NTD, p. 9-10 and Fig. 16).

2. What materials do you use to make flow guides?

We have suggested two major types of flow guides. One type (we call them super-capillary flow guides on p.2) is represented by areas of enhanced capillarity that are prefabricated by changing geometry of microfluidic chamber(s). Therefore, these guides will be made mainly from the same hydrophilic material as the chamber itself, such as glass (including quartz), silicon (Si), or silicon covered with SiO_2, etc. (p. 2-4, Figs 2b, 3a-j).

Another type (anti-capillary flow guides, ibid) is surface flow guides (Fig. 3K, L) that can be formed by hydrophobic materials on hydrophilic substrates. They can be fabricated by deposition of special films, coatings, micro- or nano-particles (diamond-like carbon on glass substrate, carbon nano-tubes on glass or on metal strips) (p.4).

3. How do you secure the flow guide (or the flow-guide materials) to the substrate?

Super-capillary flow guides are alterations in profile geometry on or in the walls that form the microfluidic chamber (p. 2-4). Therefore, they are essentially parts of the chamber formed during its fabrication, so they do not need to be specially secured. For example, flow guides can be etched on Si, glass (quartz), fused silica, etc. by using dry etching, wet etching, plasma etching; engraved with laser engraving machine on glass, carved with special carving machines, or even created manually on hydrophilic substrates (like glass) with tools like a diamond stylus. Some of protruding/projecting flow guides, such as filaments, tubes/capillaries, fibers, etc., will be either formed by mold-pressing, extrusion through a draw plate, or attached during drawing/pulling/forging from an ingot, in which cases substrate temperature will be above melting point. Therefore, these flow guides will be fused with the rest of the chip/reactor.

Surface flow guides can be made by film deposition techniques used, for example, in semiconductor industry (p. 3-4).

4. Etched flow guides: If so, give a reference. If not, I need a detailed set of instructions for how to do your "special" (i.e., not well-known) etching.

Word special does not mean not known, but rather compatible with specific materials and chip design.

5. Carved flow guides: Carved how? Can I get by with my Swiss army knife, or do I need a diamond stylus, or something even more hi-tech? Does my choice of carving implement depend upon the substrate on which I'll be carving? If so, recommend a carving implement for at least one type of substrate that I might choose.

We have indicated on p. 4 laser blasting, mechanical surface processing, such as sanding, scratching (with a diamond or sapphire stylus), or engraving. Carving can be applied to glass and Si.

6. Attaching filaments, tubes/capillaries... HOW?

Attaching filaments, tubes/capillaries can be done during capillary pulling from ingot of a special design (see e.g. Fig. 6, 7a). See also answer to point 3.

7. Soldering... HOW?

Soldering can be done by touching glass substrate under glass filament with a solder, which has tip temperature higher than melting temperature of the glass. It also can be done by laser beam focused on the area of contact between glass substrate and glass filament/tube. Another option is heating caused by friction during vibration (ultrasonic soldering/bonding).

8. Micro- and nano-particles... Made of what? Is the choice of materials dependent upon the type of nanoparticle (e.g., does a "micro-crack" imply a different material from a "bead")?

Micro- and nano particles can be tubes, beads, grinded powders made of glass, carbon, oxidized metal, and many other materials.

Choice of material depends on the purpose. If micro- and nano-particles are used for flow guiding, material must be hydrophilic (glass, oxidized metal). For surface flow guides hydrophobic micro-, nano-particles can be used (carbon) (p. 4).

9. Sandwich layers. How do I make these "sandwiches"?

Sandwiches can be made of glass frames (Fig. 3j) , or layers of Si substrate, glass frame, and glass cover slip, assembled and held together either by gluing, ultrasonic soldering (bonding), lapping, polishing (p. 4).

10. Flow guide geometry. Is the fabrication technology the same for each of these geometries? How would I choose among the possible geometries to best achieve the invention?

Choice of geometry depends on technology available. Fig. 12a,b present optimum geometry for chip-on silicon, Fig. 12c,d present a preferred design for chip-on-glass.

11. Varying the degree of capillarity. How do I control the degree of capillarity?

The degree of capillarity can be controlled by controlling say a shape of the flow guide (e.g., angle and depth of the Small volume (sample) holding flow guide on Fig. 4), material it is made of.

12. Varying the volume of flow. How do I control that?

Varying the volume of flow one can by varying flow guide volume or its cross-section profile along its length

13. Holding the flow. How do I do that? How do I control it so that I can vary the "holding" of the flow? Is there a formula I can use to calculate how much to expand the guide's profile depending on how much fluid I want to hold? If not, is there a simple way of creating a "calibration curve" empirically?

We are not talking about holding the flow. We say "flows along OR held at", referring to the two functional types of the flow guides - (i) large volume filling and (ii) small volume holding ones. Liquid flows along (i) when it fills the chamber, and liquid is held at/on (ii) before the chamber is filled. Generally, a holding guide is made by creating an isolated area (an island) of increased (or decreased) capillarity (Fig. 2c, 3L). Volume of the held liquid depends on geometry of the holding guide. If shape of the held liquid is known, its volume can be easily calculated using simple known formulas (for, e.g., cylinder, prism, pyramid, etc.). Calibration of holding areas can be made by placing known volume of liquid in the area and finding a maximum volume the holding area can hold (otherwise, the liquid to be held will leak from the holding area.

14. Flow guide fabrication: You refer to "special" films, coatings, etc. What are some specific examples of such films, coatings that would be relevant for my purposes.

Special films can be say nano-films of some composition or geometry relevant to our purpose, which can be found in future.

15. Does your "deposition" process depend upon the film or coating material I choose? The substrate on which I wish to deposit it? Other factors such as the geometry, shape, space available for deposition, etc.?

It is well known to those skilled in the art that "deposition" processes and regimes may depend on the film or coating material, type of the substrate, and many other factors such as the geometry, shape, space available for deposition, etc.

16. You mention also sand-blasting, laser burst and other techniques. Are these well-known in the art? If possible, specifically refer me to a paper where sand-blasting of micro-chips is described.

Sand-blasting, laser burst, laser engraving and carving are well known, widely used in industry for glass, metal, ceramics, plastic processing.

Sand-blasting of micro-chips is described in:
Yoshikuni Kikutani, Manabu Tokeshi, Kiichi Sato, and Takehiko Kitamori1, "Integrated chemical systems on microchips for analysis and assay. Potential future, mobile high-performance detection system for chemical weapons", *Pure Appl. Chem.*, Vol. 74, No. 12, pp. 2299–2309, 2002. © 2002 IUPAC

17. How would I go about selecting a useful hydrophilic material to make the invention? How would I go about selecting a useful hydrophobic material to make the invention?

Selecting hydrophilic and hydrophobic materials includes their compatibility with:

- chip fabrication technologies,
- chemical and biological reaction to be carried out on chip
- experimental measurements of desired flow-guiding properties

18. You identify "ports' as "specially designed openings." Special in what ways?

Design of ports must provide at least the following:

- Easy access to designated flow guide
- Prevent loading tool (say pipette) from touching anything but the designated flow guide.
- Loading ports can be coated with hydrophobic material to prevent unwanted capillary action

19. Do the specific design elements vary as a function of the port's purpose (loading vs. venting)? What are the critical design elements and how do I choose among them to fabricate specific types of port? How should the selected design elements be put together to give me the type of port I need?

They do. See the answer above (point 18). Design also depends on specific loading tools. In our sketches we assumed the use of our loading device (see answer 1)

20. Sealants for ports. You mention PCR oil. Is that a well-known material?

PCR oil is well known material used in industry. We can also use films made of polyethylene, Teflon, etc.

21. I need guidance from you on which polymer films to choose as sealants.

The most widely used is parafilm (sold by several companies).

22. Electro-kinetic injection. I need to read about this technique -- give me a good reference.

http://www.elsevier.com/wps/find/bookdescription.cws_home/523599/description#description

23. On page 7, you mention "specially" designated (designed?) large volume liquid port and large volume flow guide. What is "special" about this port and flow guide? I'm afraid I might not get the invention right if I don't understand what "special" means here.

The port intended for loading large volume liquid component into large volume flow guide

24. How do I make a "vibration by pass membrane"? How do I make it work?

Those skilled in the art know.

25. Where can I get a "micro-pump"? How do I attach it to the chip?

There is whole variety of commercially available micro-pumps and appropriate tubing for their c

26. Is there an example of "porous" thin wall in the literature? How do I make the pores hydrophobic inside?

There are glass processing procedures which are known. Depending on the material pores can be hydrophobic.

27. If the pores are hydrophobic inside, will just an air puff actually move liquid through the pores?

Yes.

28. The stirrer-straw also sounds great. Wouldn't I need a very large magnetic field to get any momentum transferred to such a tiny magnetic element? How do I go about attaching the stirrer element at only one end?

As in existing laboratory stirrers, the magnet will be outside of the chamber, so it's size does not place any constrain.

29. The nanomagnets/nanodipoles also sound intriguing. I'm worried about risking the time and effort on developing that approach to reagent mixing. Is there a reference in the literature that assures me that I can move such tiny magnets/dipoles around in a fluid using externally applied fields?

Yes. Similar mechanisms work in liquid crystal displays (LCD).

30. Do "bobbin-mounted" flexible capillary systems exist?

The loading system we proposed is original. However one can purchase flexible capillary on bobbin (http://www.polymicro.com/

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Fig.1. Bubble-less filling of a capillary with filament

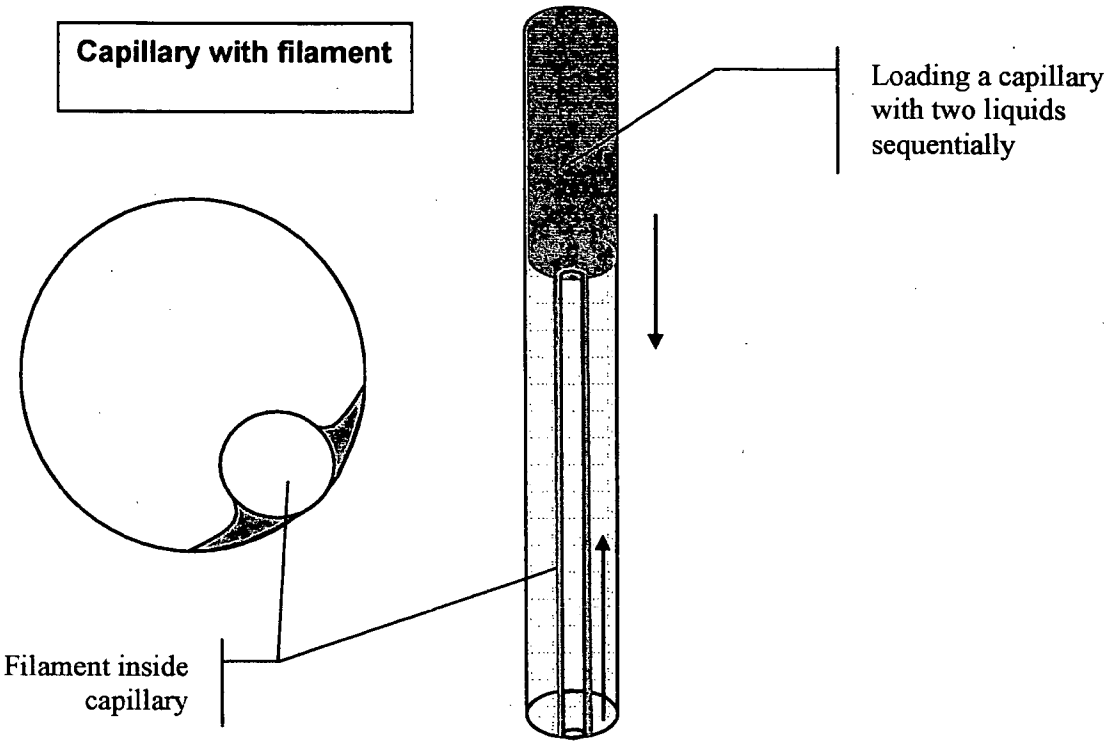


Fig. 2a. Loading liquid component into chamber/channel of the microfluidic chip using pipette or capillary dispenser

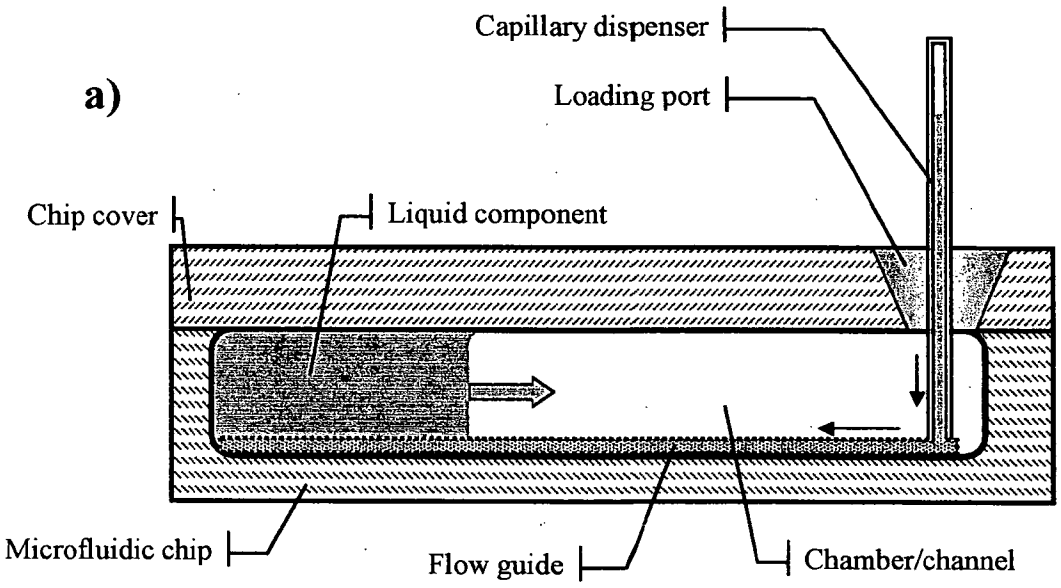


Fig.2b. Saggital cross-sections of microfluidic chambers or capillary channels with a flow guides.

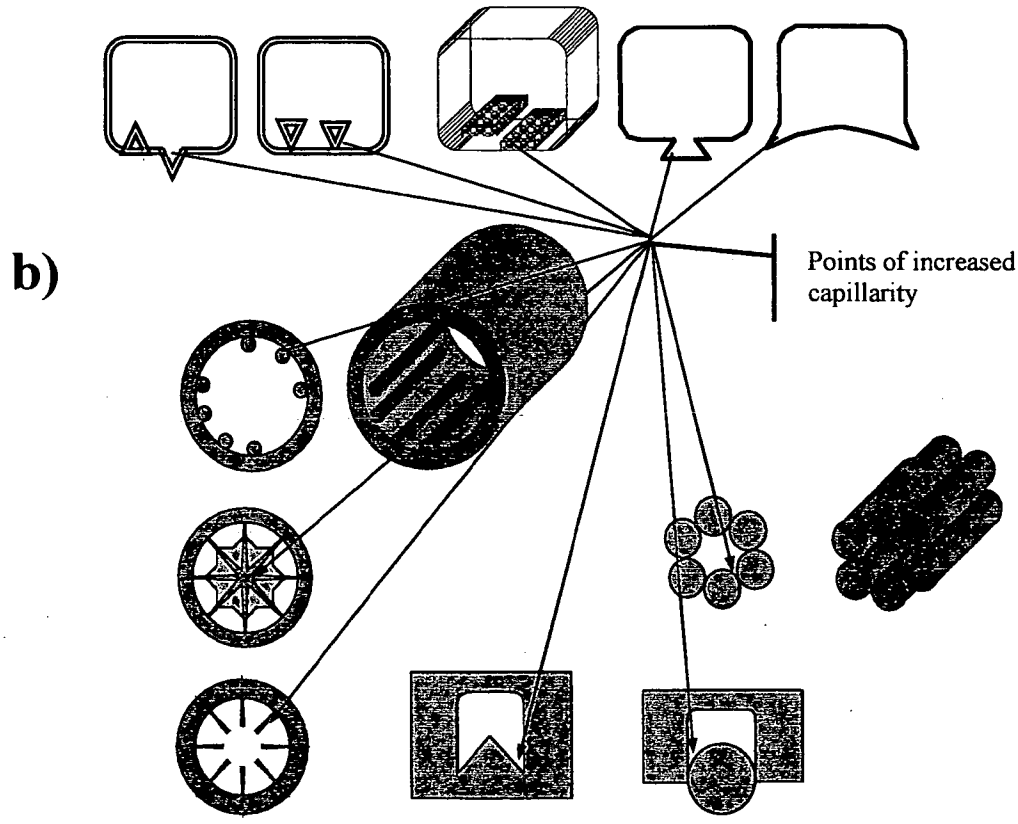


Fig. 2c. Micro-channel with loading ports and two flow guides for loading and mixing two liquid components (c)

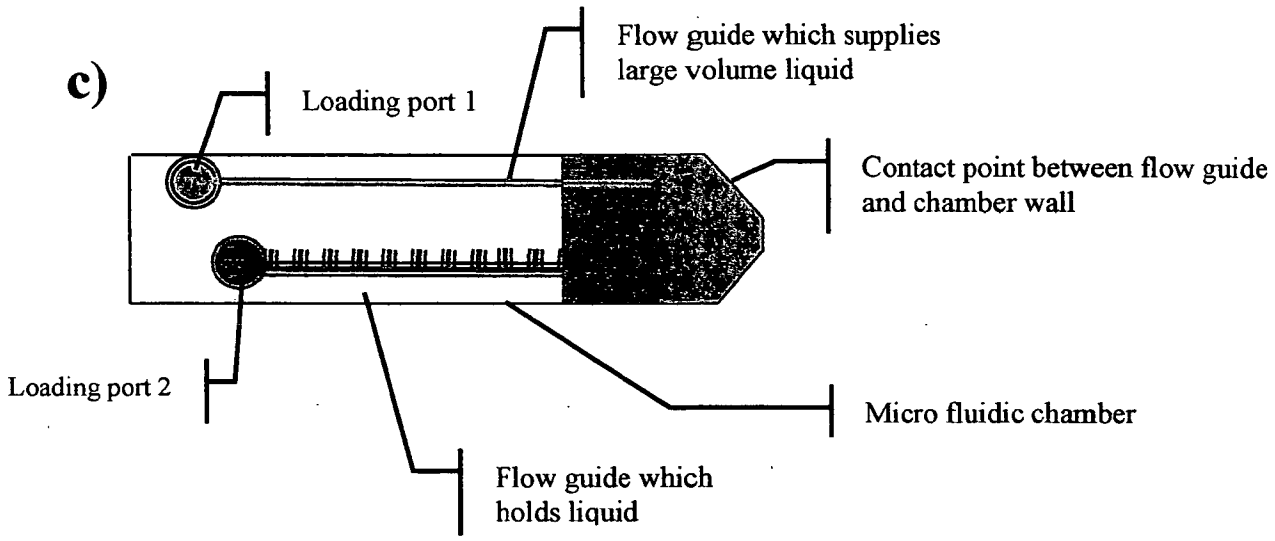
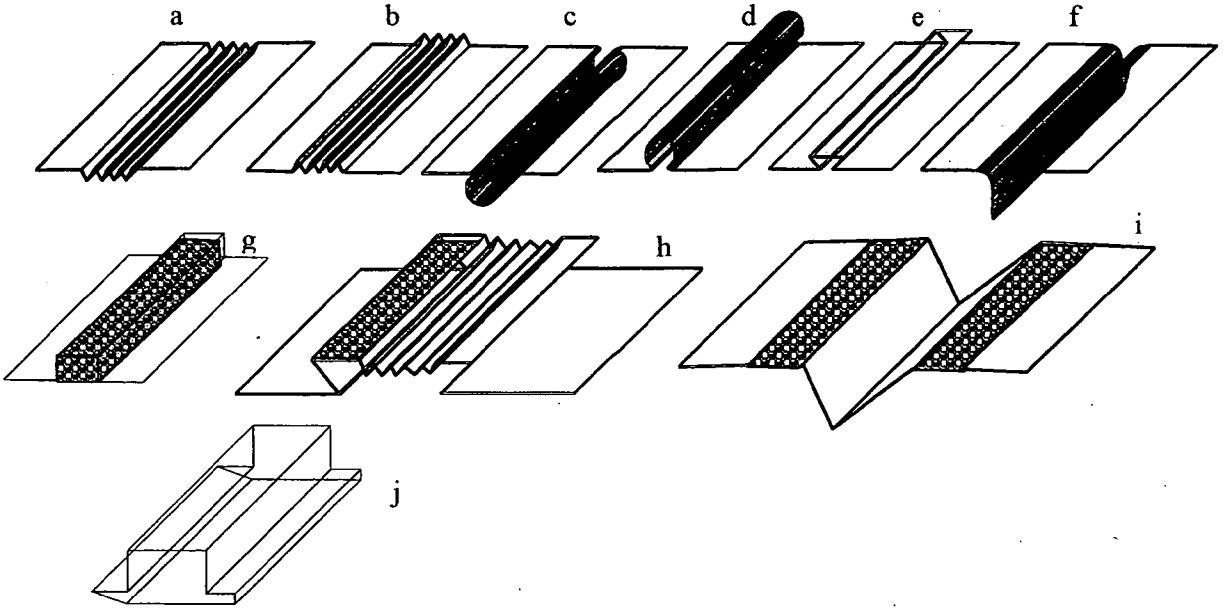


Fig.3. Flow guides of different types.



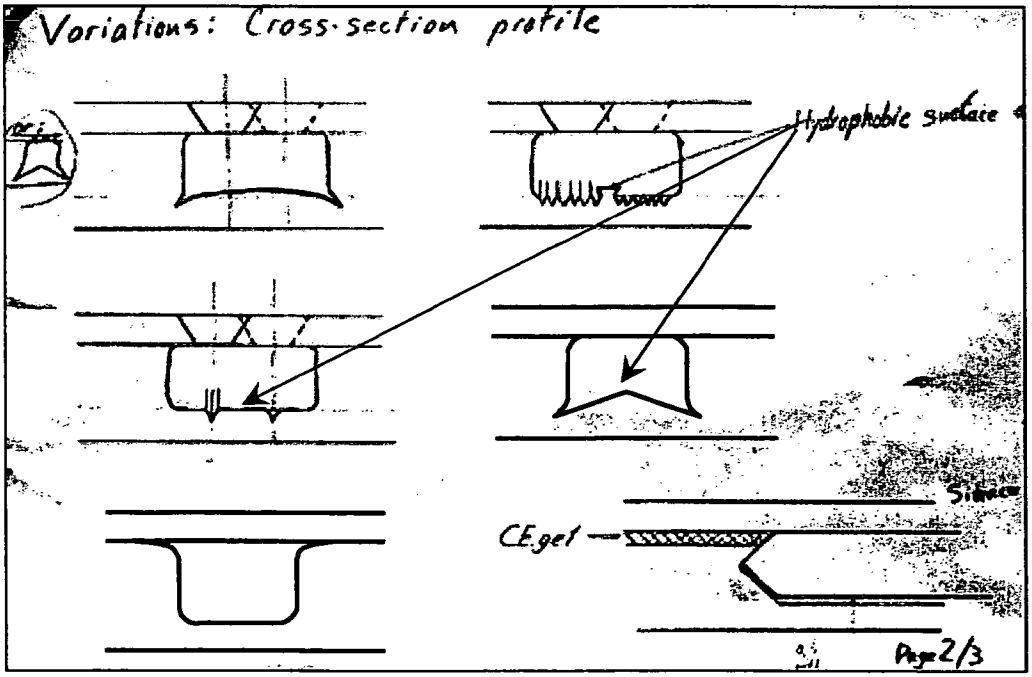


Fig. 3K. Surface (flat) flow guides created on chip by forming and combining areas of enhanced and reduced capillarity

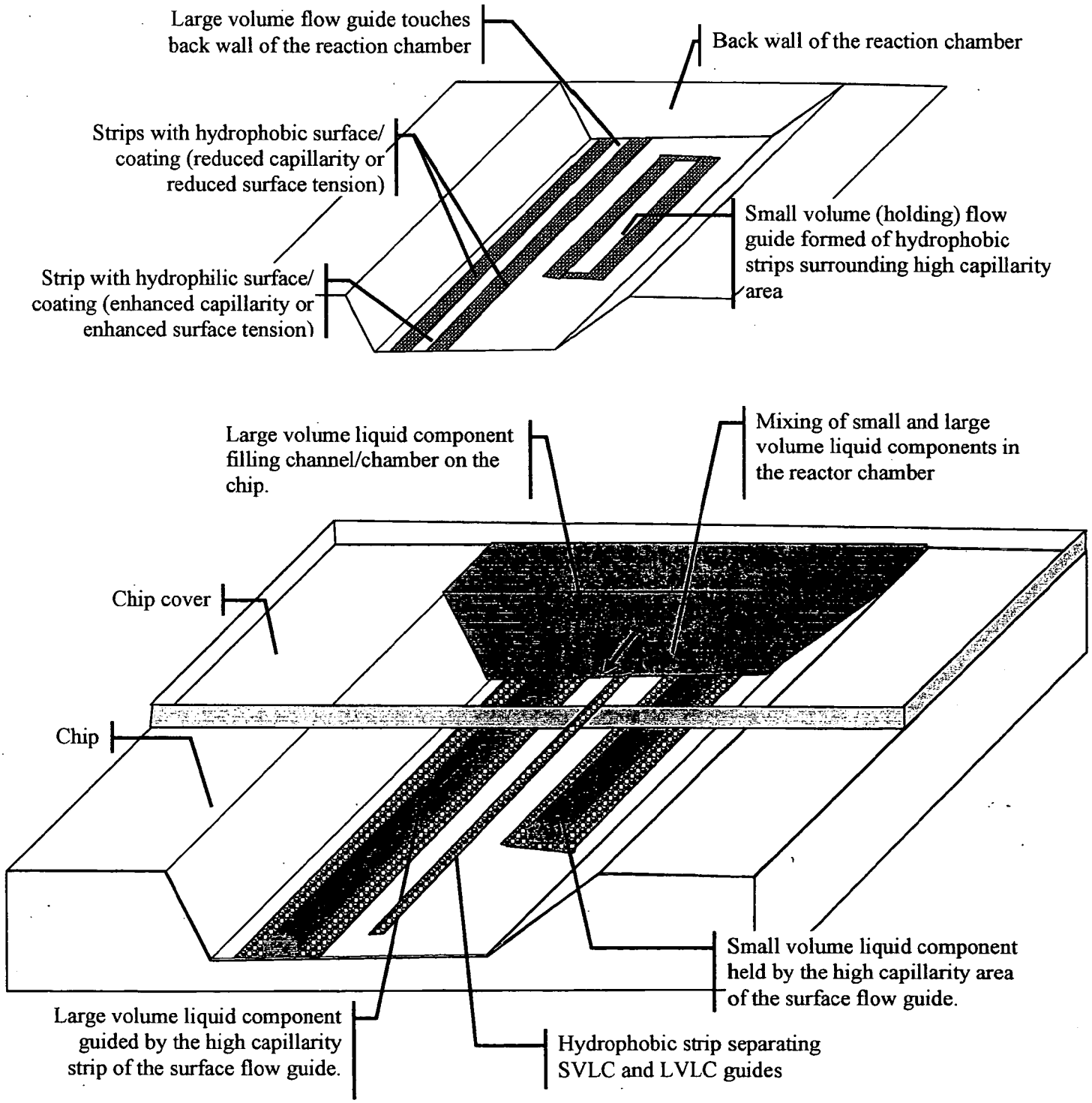


Fig.3L. Possible configurations of surface flow guides created on chip by forming and combining areas of enhanced and reduced capillarity (arrows denote flows of liquids)

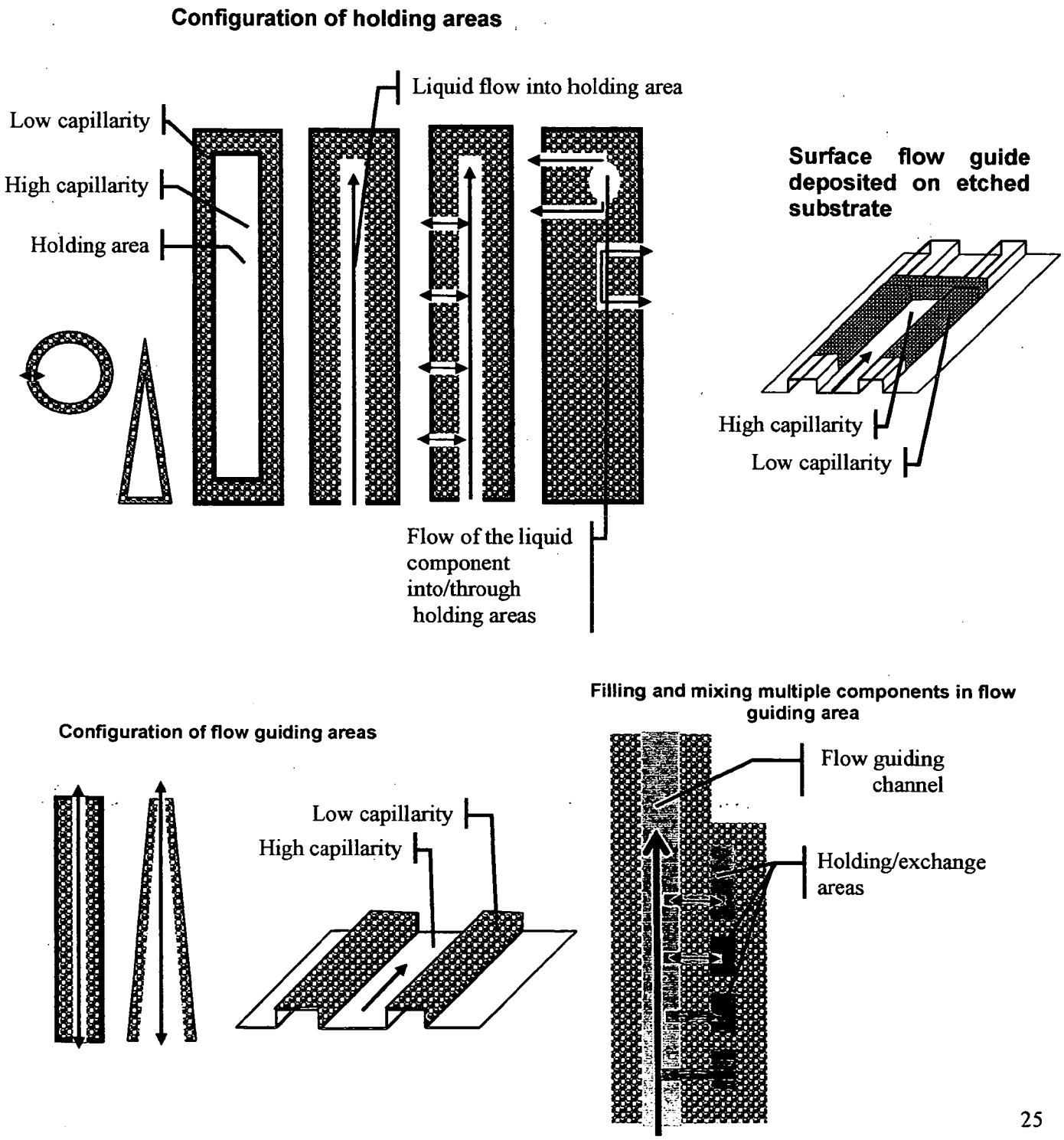


Fig. 4. Loading ports (openings) for filling Microfluidic chamber

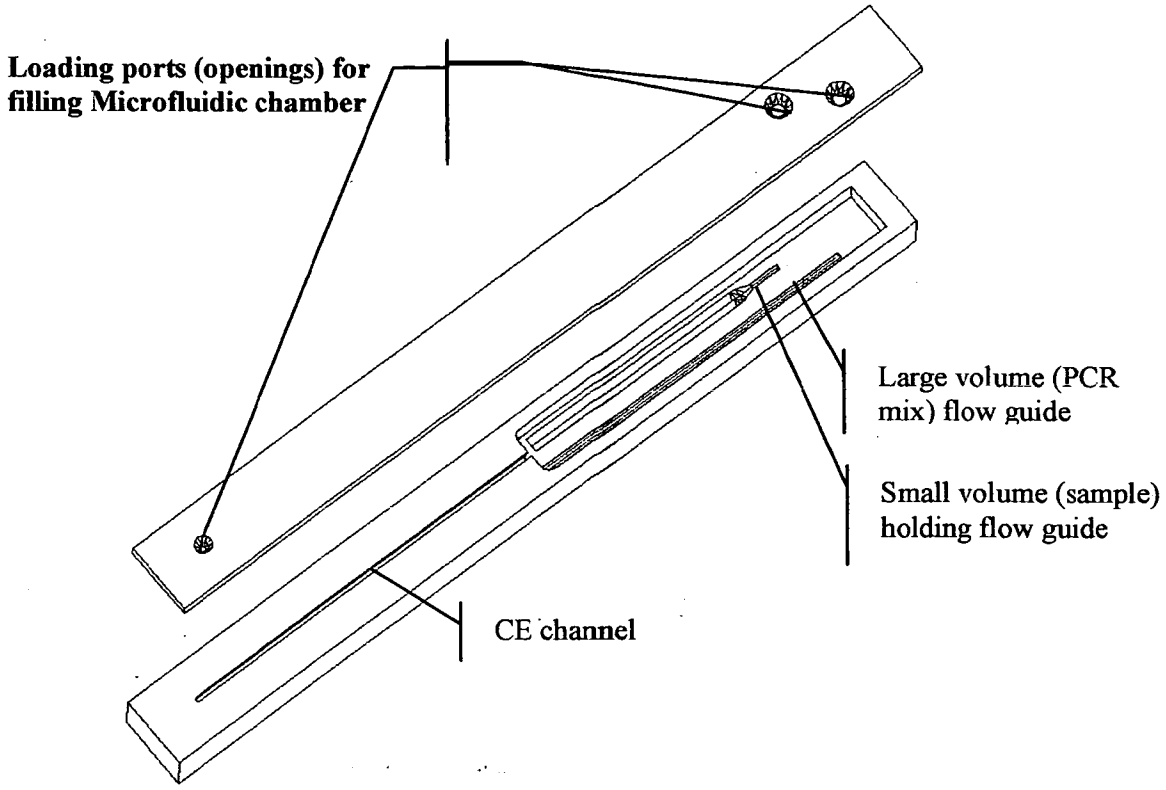


Fig. 5a. Microfluidic chambers assembled from many capillary tubes.

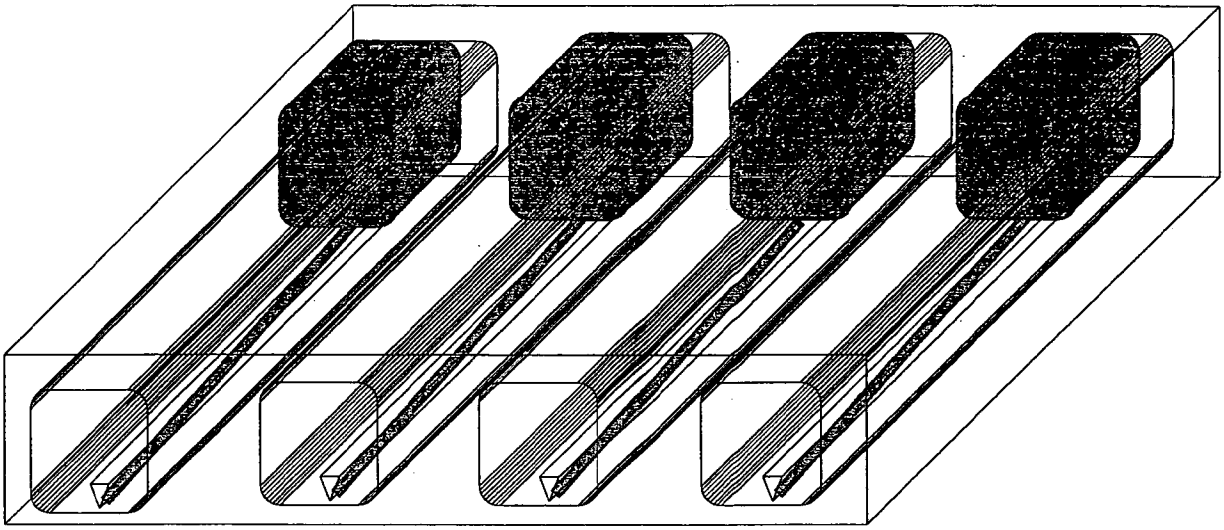
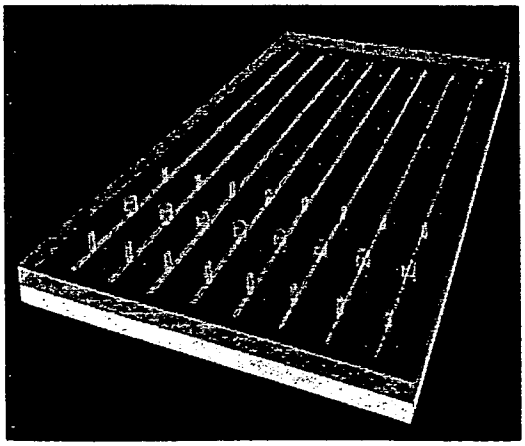


Fig.5b. Microfluidic chambers manufactured by scalable microfabrication with etching on glass, or silicon, or plastic, or combined chip (plate).



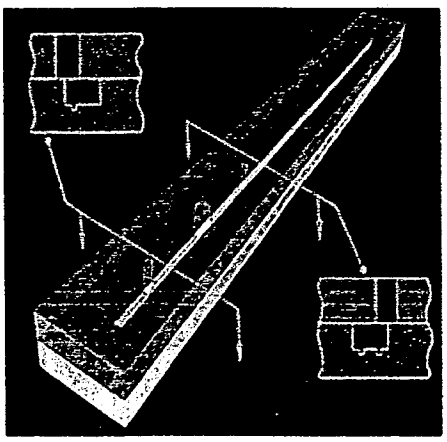
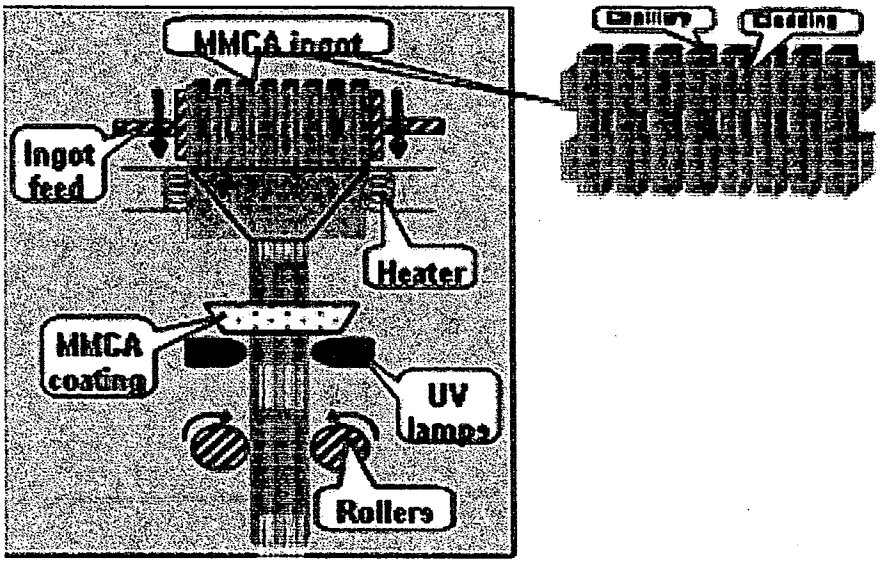
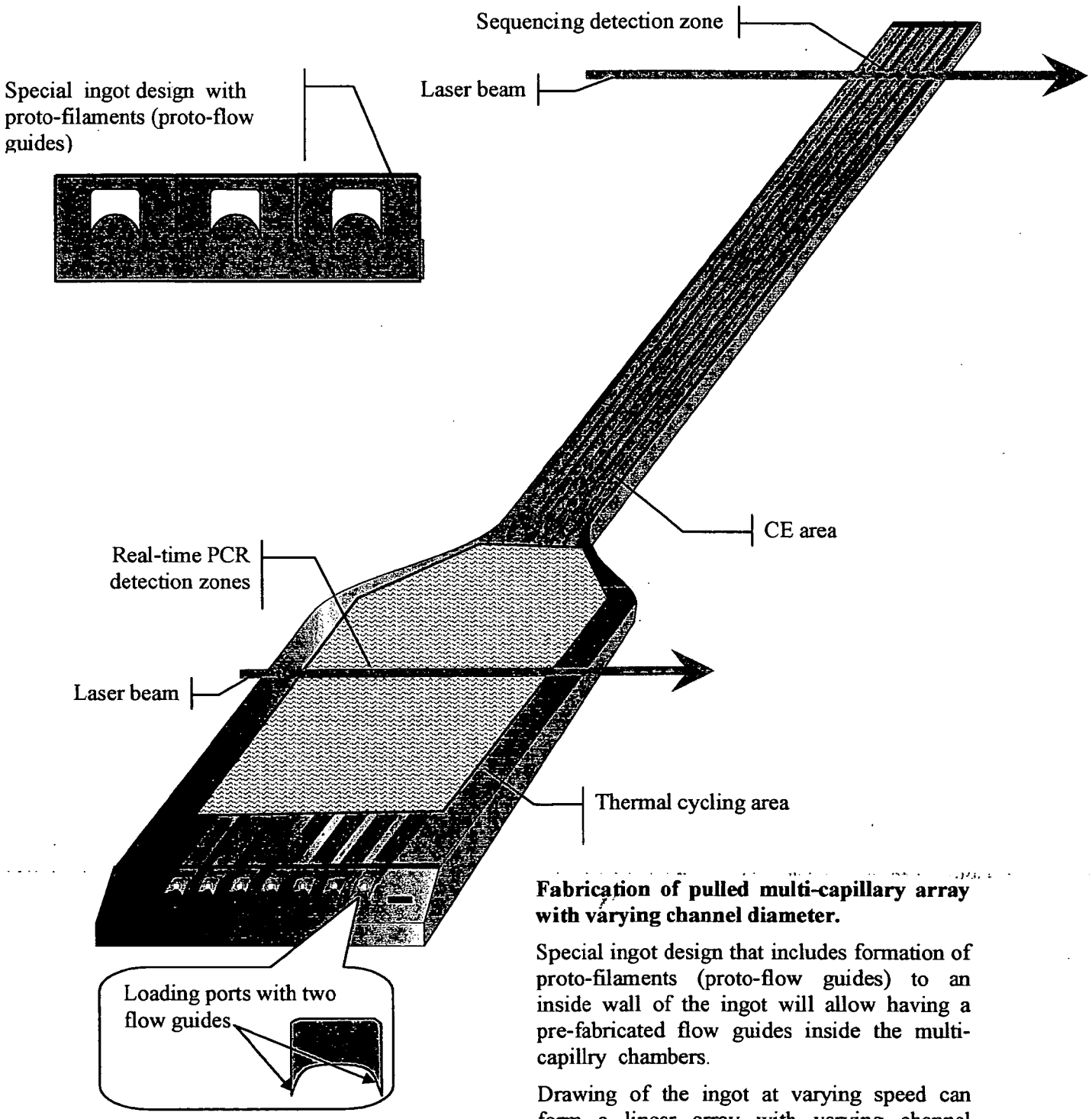


Fig. 6. Fabrication principle of multi-capillary PCR/CE array



Fabrication principle of multi-capillary PCR/CE array with split capillary ends.
In the proposed fabrication process both inner and outer ingots are made of the same type of glass, but the outer cladding ingot is shorter than the capillary ingot. During the pulling process the outer cladding is formed only on a partial length of the array. The length of the monolithic segment is proportional to the ratio of the lengths of the cladding/capillary ingots. In the process of fabrication the structure undergoes a high-pressure treatment that aligns the centers and reduces the non-uniformity and eccentricity of the capillaries. Being monolithic, the array acts as a low-loss medium for the propagation of light. Its remarkable optical properties ensure uniform illumination of all capillaries. The production process allows formation of extremely regular arrays of square or rectangular capillaries with virtually ideal translational symmetry. This facilitates focusing of the laser beam on the center of the capillary. Very significant advantages of the proposed technology include its low cost and the absence of any specially adjusted parts in the detection zone.

Fig.7a. Monolith milti-capillary PCR/CE array with varying channel cross section pulled from ingot

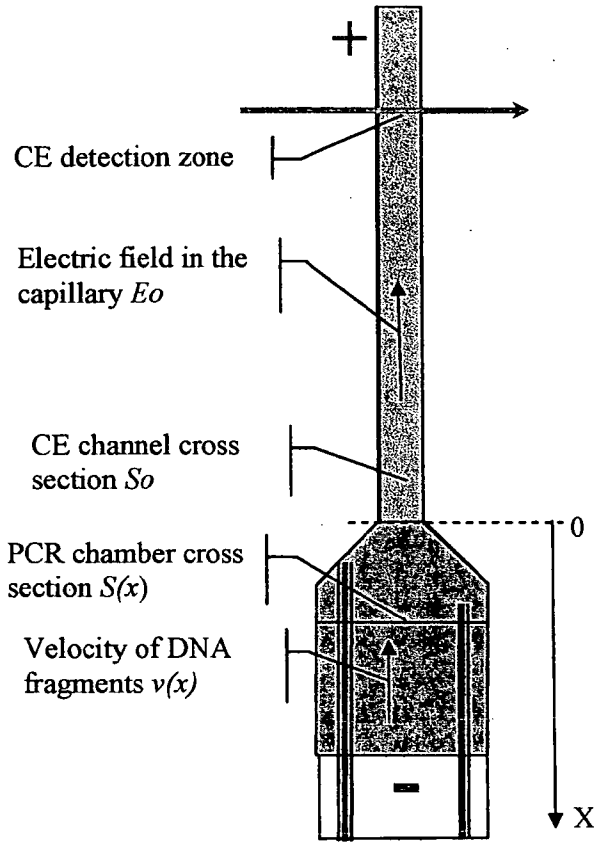


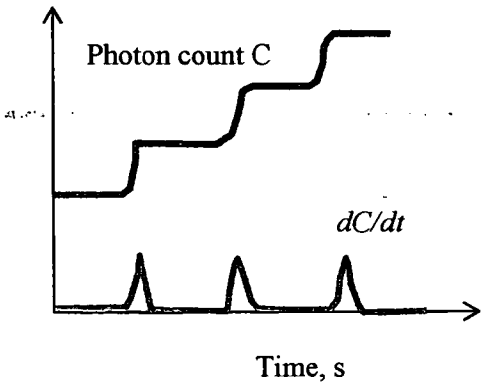
Fabrication of pulled multi-capillary array with varying channel diameter.

Special ingot design that includes formation of proto-filaments (proto-flow guides) to an inside wall of the ingot will allow having a pre-fabricated flow guides inside the multi-capillry chambers.

Drawing of the ingot at varying speed can form a linear array with varying channel

Fig.7b. One channel of the monolith milti-capillary PCR/CE array





Injection and sequencing of DNA sample from the PCR chamber into CE channel can be done using either special 3-electrode system or can be carried out as injection from an " unlimited" source of DNA target. In this case for x = 0, electric field $E=E_0$.

Let us consider dependence of $E(x)$. $E(x)=\frac{d\phi}{dx}$, where ϕ is electric potential. According to Ohm's law, $d\phi=I\times dR$, where I is electric current which does not depend on x, and

$$dR = \frac{dx}{\sigma(x)S(x)}$$. Therefore For x<0 electric field

$$E(x) = I/\sigma(x)S(x) = E_0\sigma_0S_0/\sigma(x)S(x).$$

Therefore, velocity of DNA fragments of length i in the PCR chamber will be:

$$v(x) = \mu_i \times E(x) = \mu_i \times E_0\sigma_0S_0/\sigma(x)S(x).$$

During the observation time between t_0 and $t_0+\Delta t$ we will collect photons from N DNA fragments which at the beginning of the injection were located between x_0 and $x_0+\Delta x$.
Since $N=n(x\times)V$, where $n(x)$ is fragment concentration in the PCR area and $V=S(x_0)\times \Delta x = S(x_0)\times v(x_0) \times \Delta t = \mu_i E_0 \times S_0 \times \sigma_0 \times \Delta t$.

THUS, the photon count $C(t)$ which is observed at the CE detection zone (Fig. 7b) does not depend on the cross section of the PCR chamber and will change in time as a step-like function according to time needed for i-type DNA fragment having mobility μ_i to reach the detection zone (see graph in Fig.7b)

Fig. 8. Pre-fabricated optics on/at microfluidic chamber.

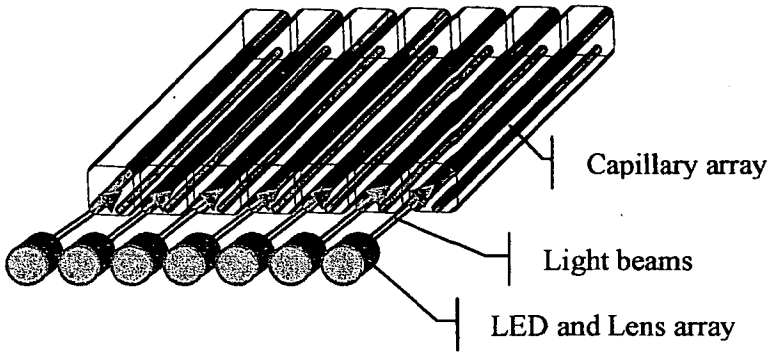


Fig.9. PCR/CE Reactor assembled from arrayed capillaries and PCR tubes with flow guides.

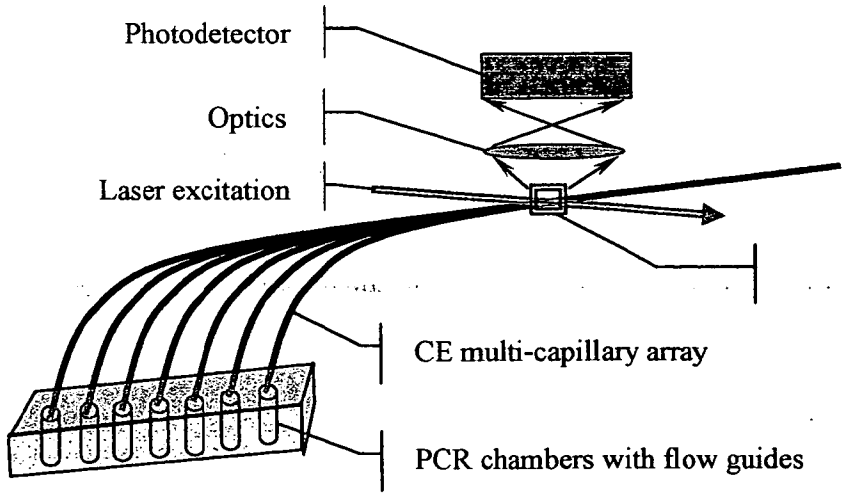


Fig.10. Integrated PCR/CE array fabricated on chip.

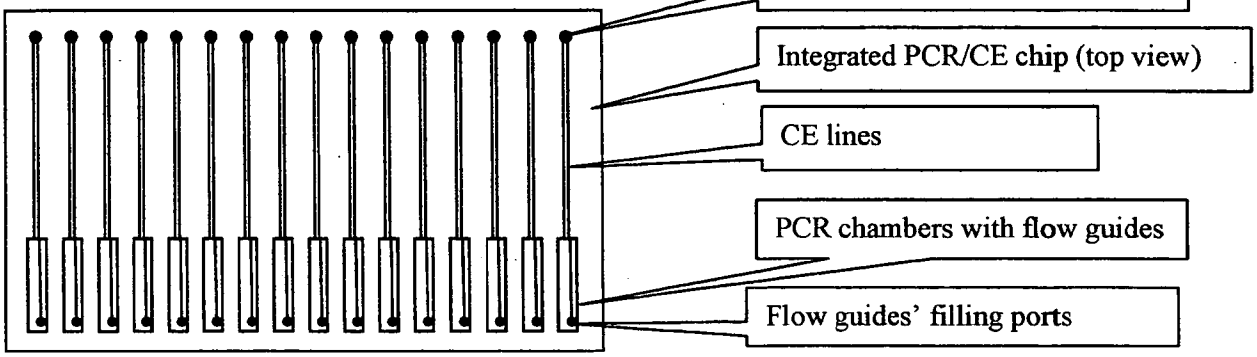


Fig.10a. Integrated PCR/CE array fabricated on chip.

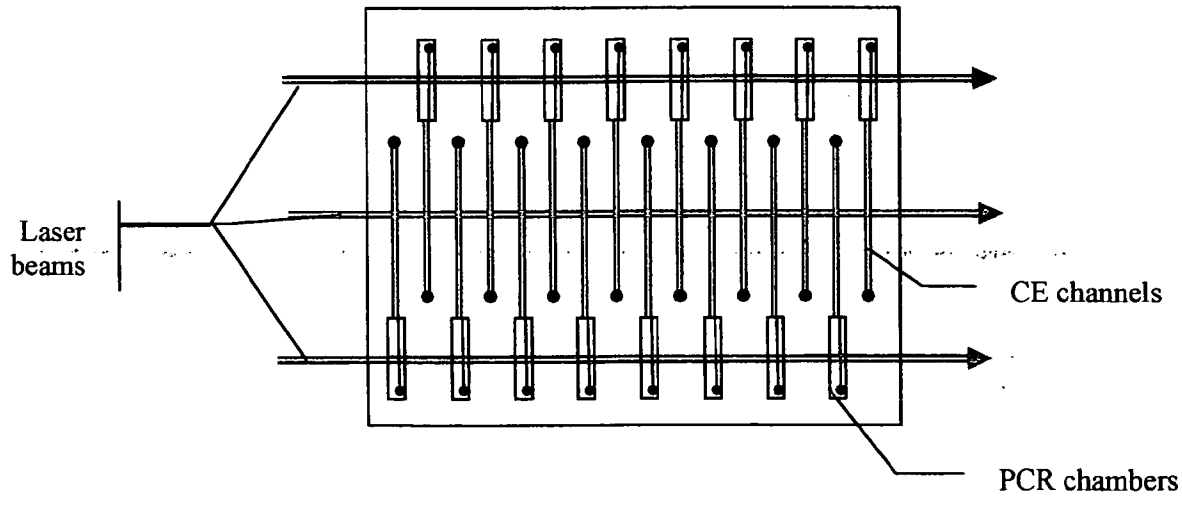


Fig.11a. Filling microfluidic chamber with two liquids via two flow guides

Top view

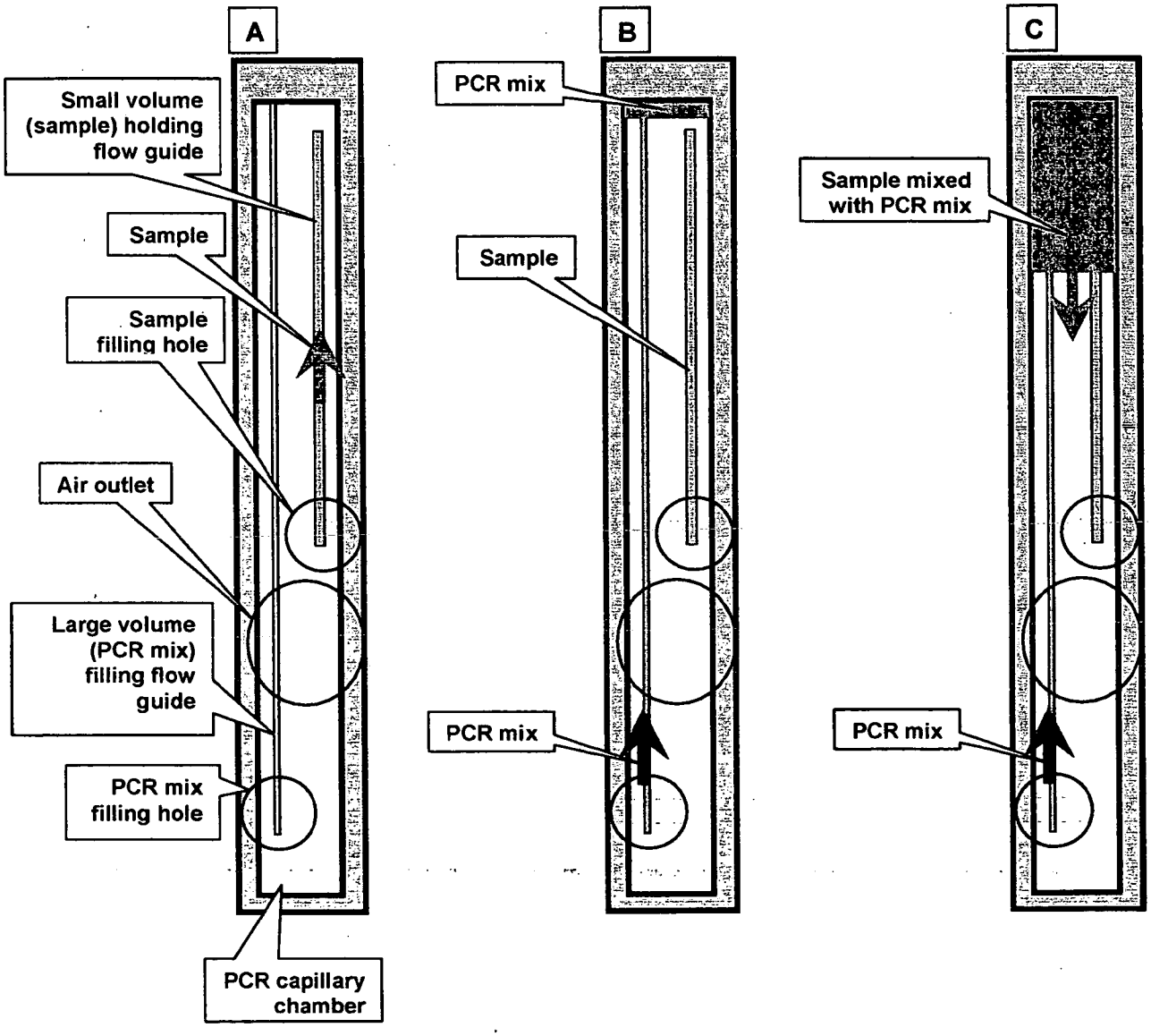
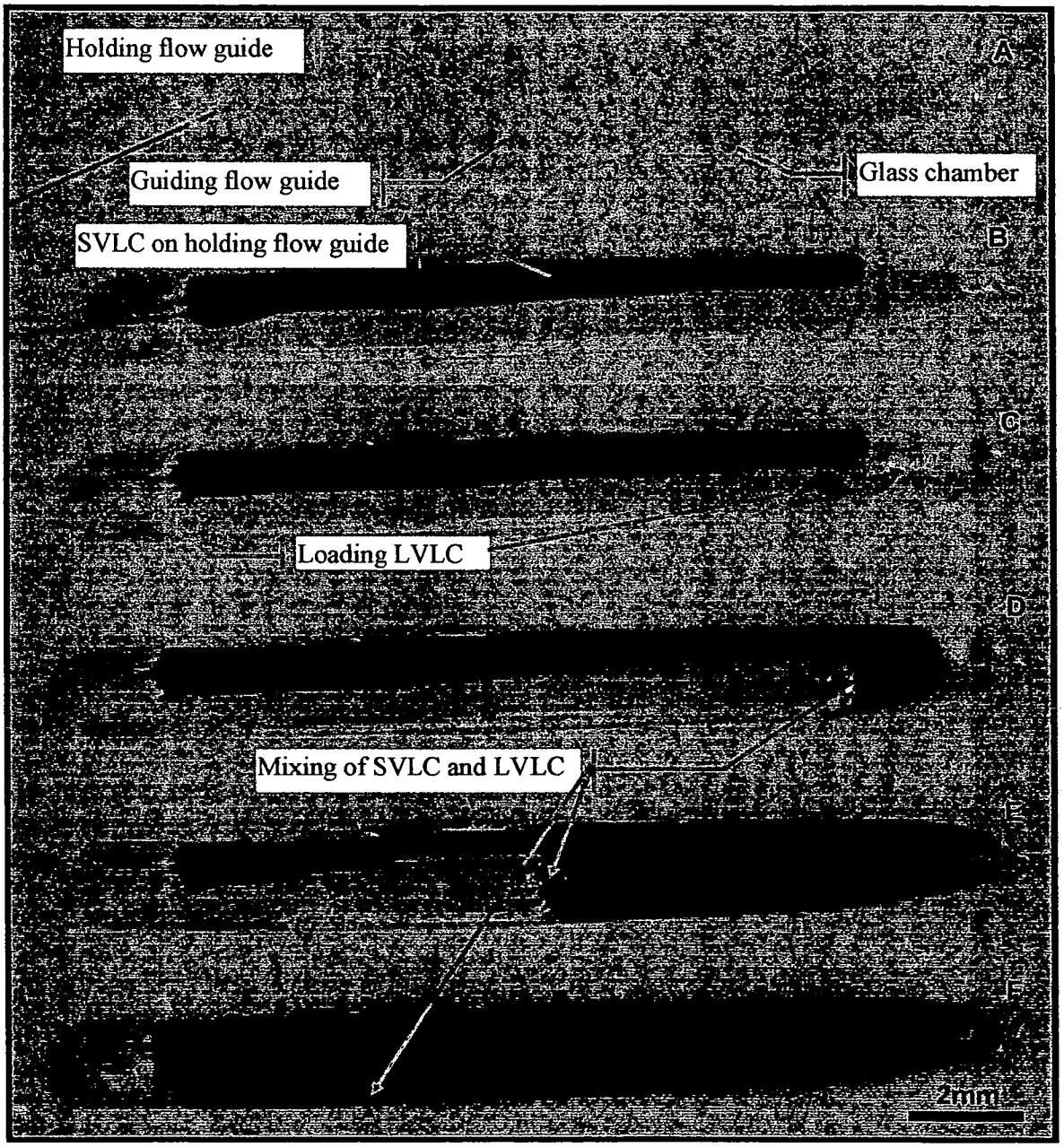


Fig.11.b. Loading two liquid components into a glass chamber with two flow-guides (holding and guiding)



We have fabricated a glass chamber with two flow guides. Holding flow guide was made of thin capillary **(A)**. It did not touch the distal chamber wall and was used for holding a small volume liquid component (SVLC, dark blue) **(B)**. Another flow guide which touched the distal chamber wall was used for bubble-less filling of the chamber with large volume liquid component (LVLC, Clear) **(C)**. We also observed a mixing of LVLC and SVLC which occurred along the length of the holding flow guide while the LVLC was moving towards the chamber inlet **(D-F)**.

Fig.11.c Bubble –less loading of two liquid components into a glass chamber with one flow-guide

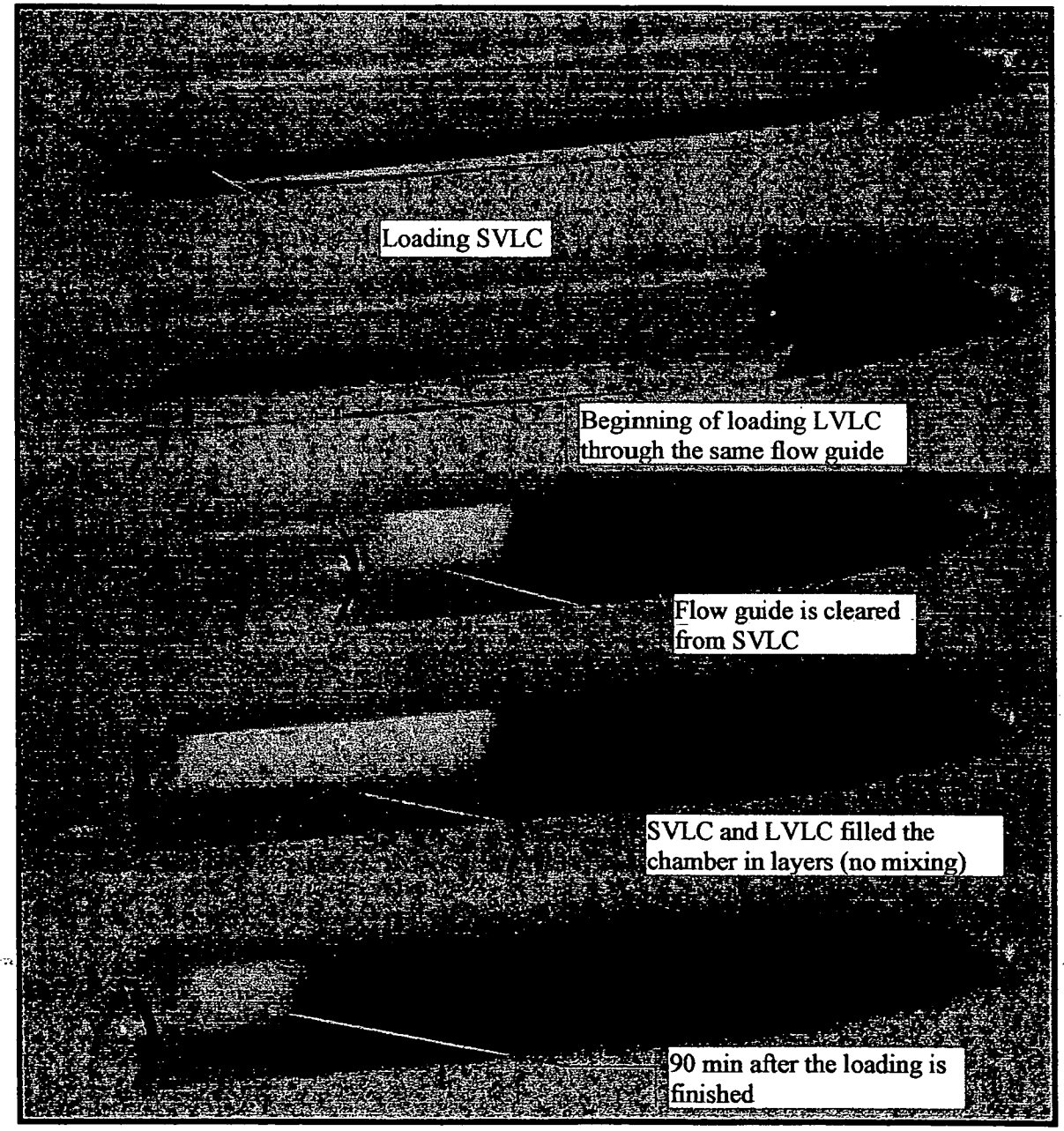
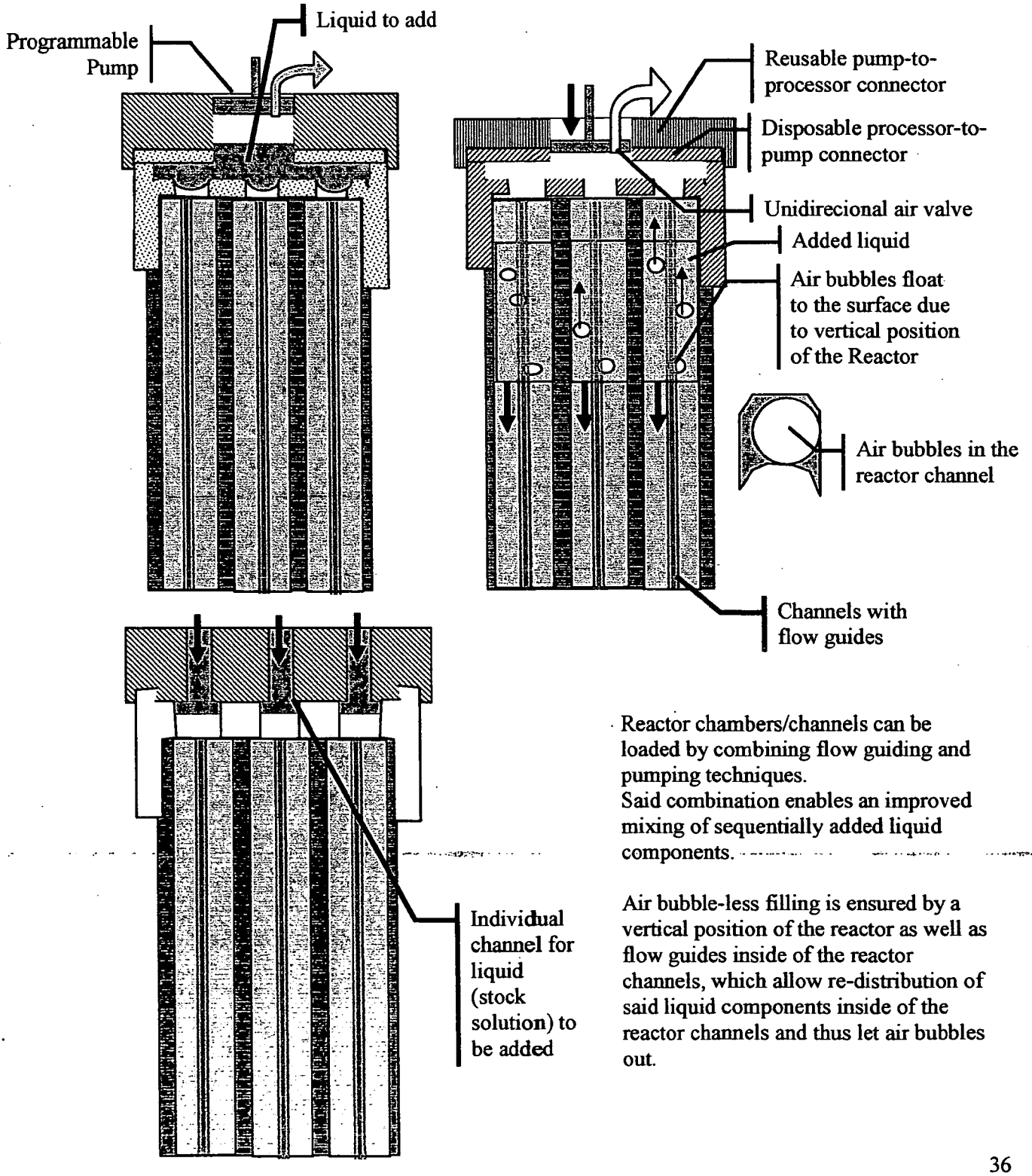


Fig.11c. Combined flow guide and pumping Reactor filling



Reactor chambers/channels can be loaded by combining flow guiding and pumping techniques.
Said combination enables an improved mixing of sequentially added liquid components.

Air bubble-less filling is ensured by a vertical position of the reactor as well as flow guides inside of the reactor channels, which allow re-distribution of said liquid components inside of the reactor channels and thus let air bubbles out.

Fig.12a. Preferred embodiment 1 (PCR/CE silicon chip)
(see Fig. 4 for 3D view and legends)

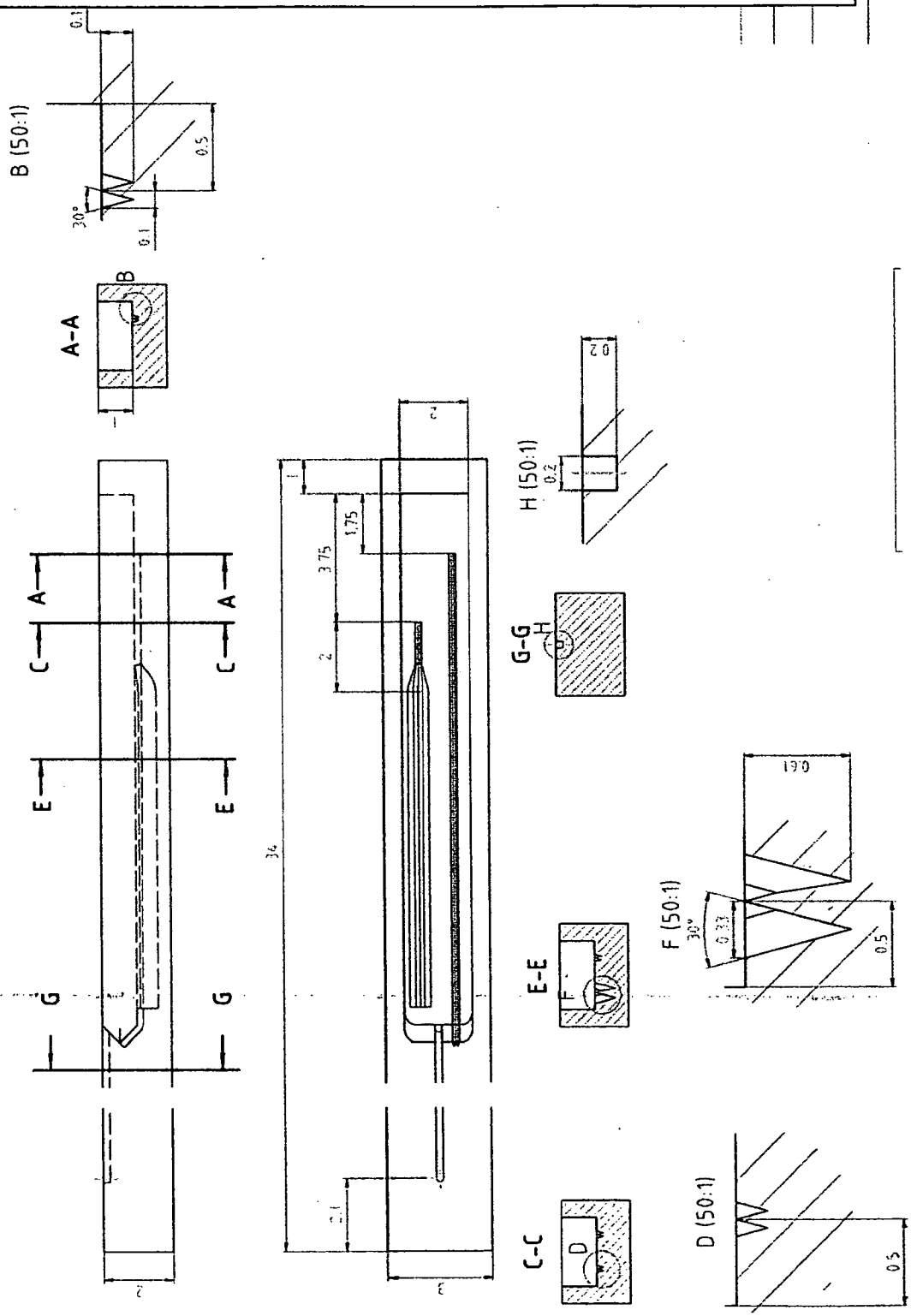


Fig. 12.b Preferred embodiment 1 (PCR/CE silicon chip cover)

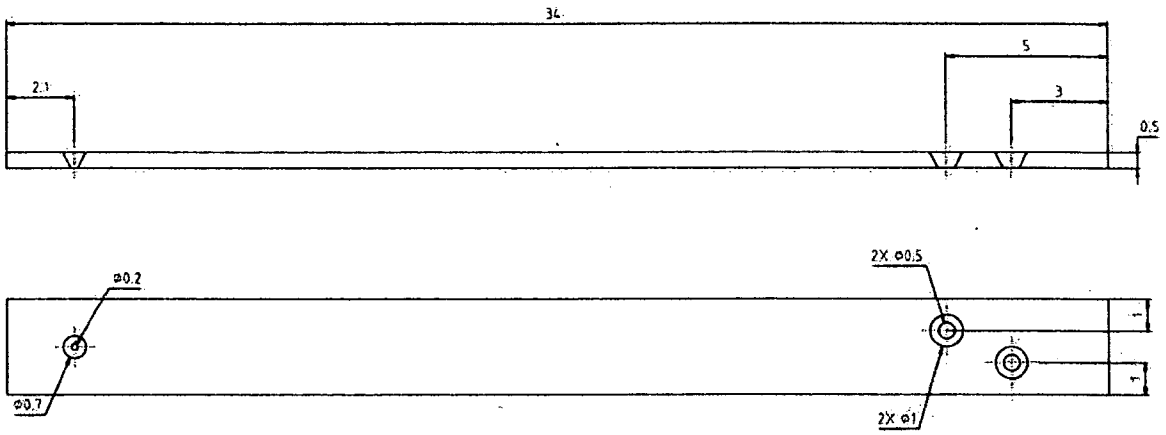


Figure 12.c. Preferred embodiment 2 for PCR/CE chip fabricated on Glass

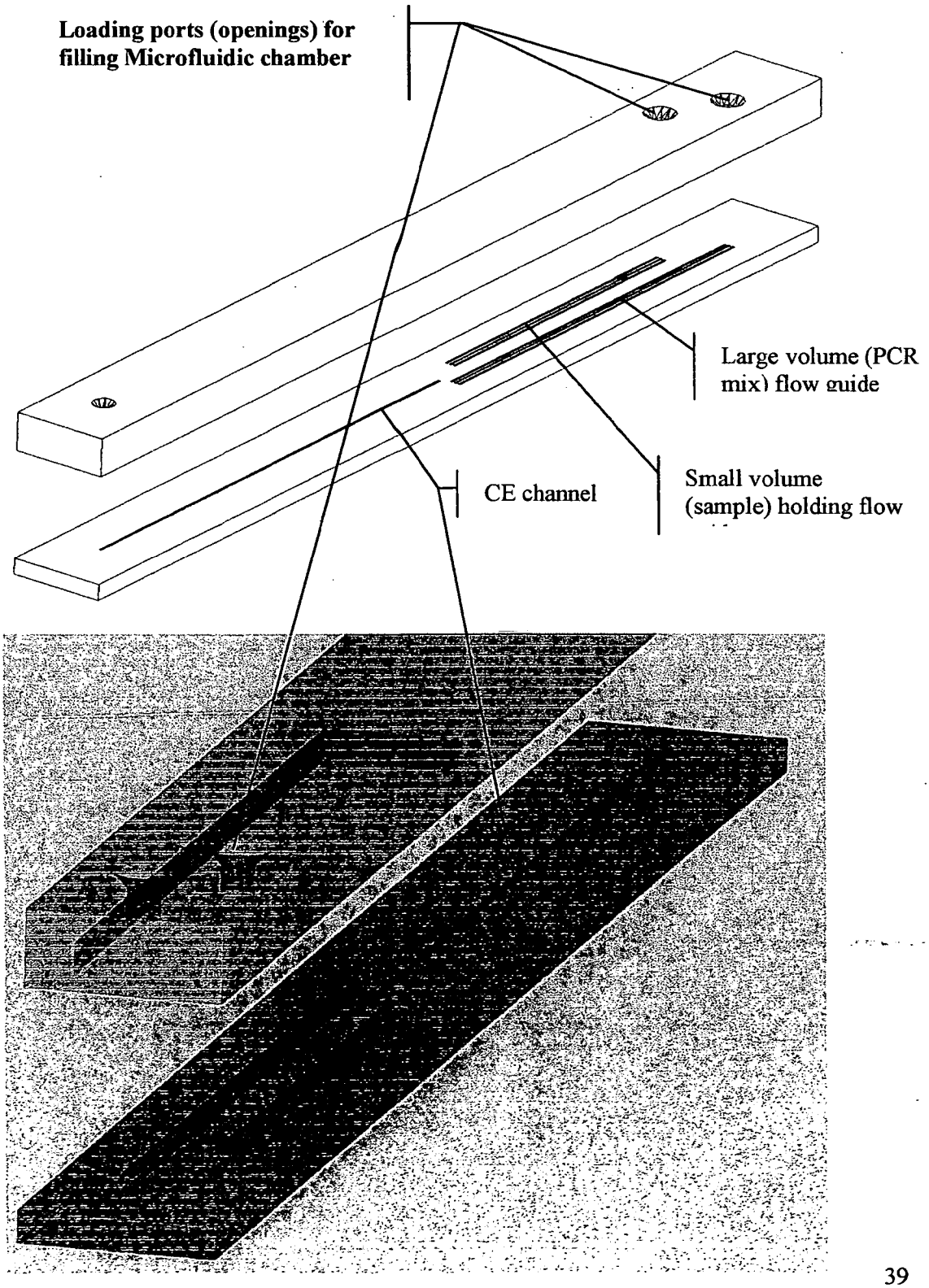
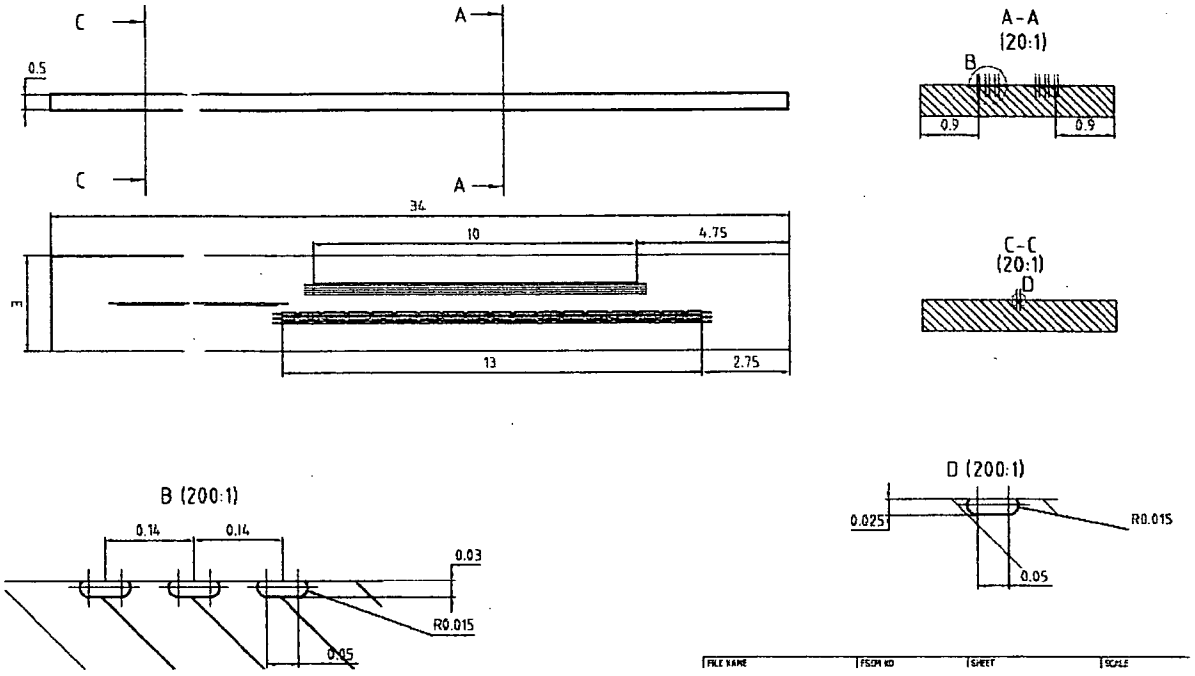


Figure 12.d. Drawing of the preferred embodiment 2 for PCR/CE chip fabricated on glass.

Chip base



Chip cover

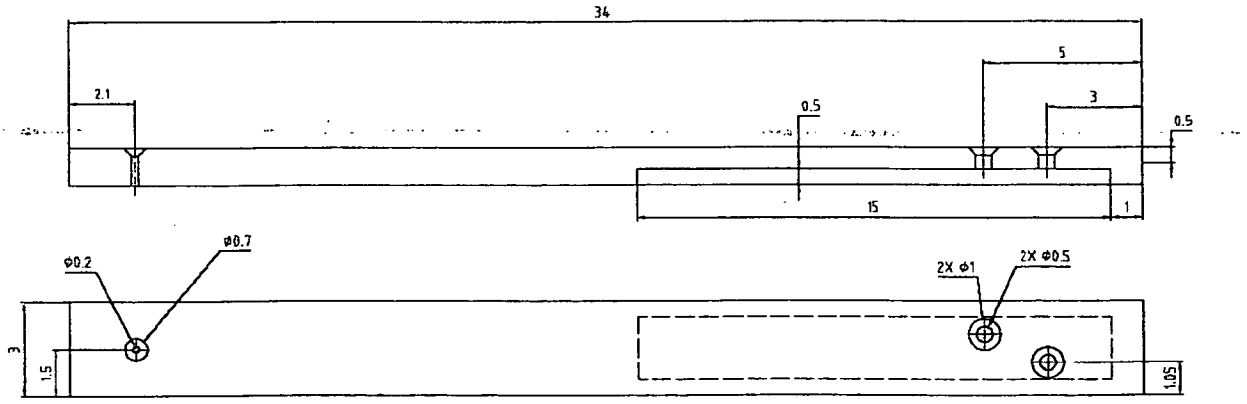
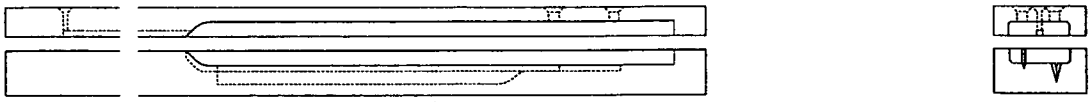


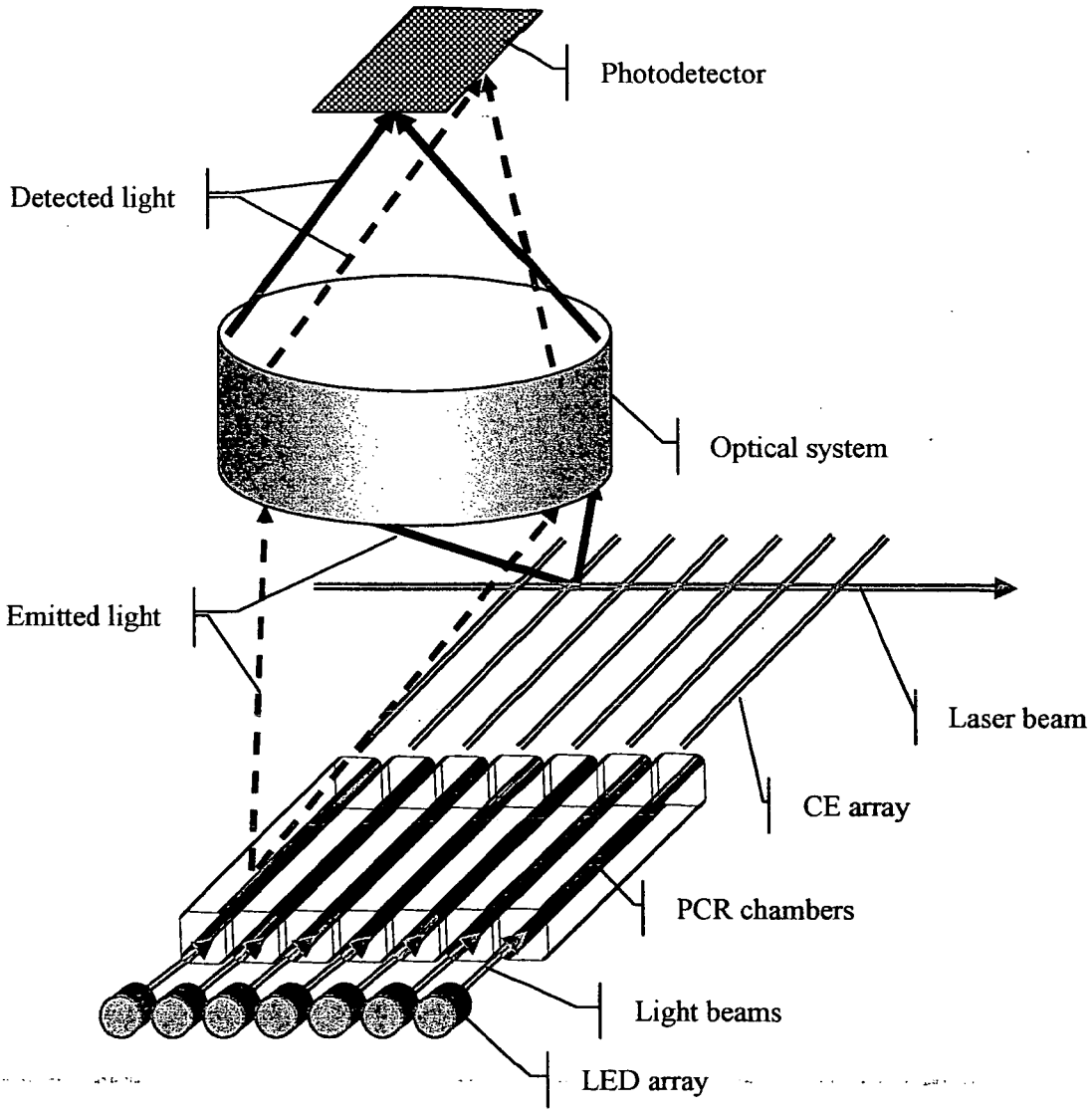
Fig. 12 e. Preferred embodiment 3. PCR-CE glass chip fabricated by combination of mold pressure, etching and laser blasting

Side view



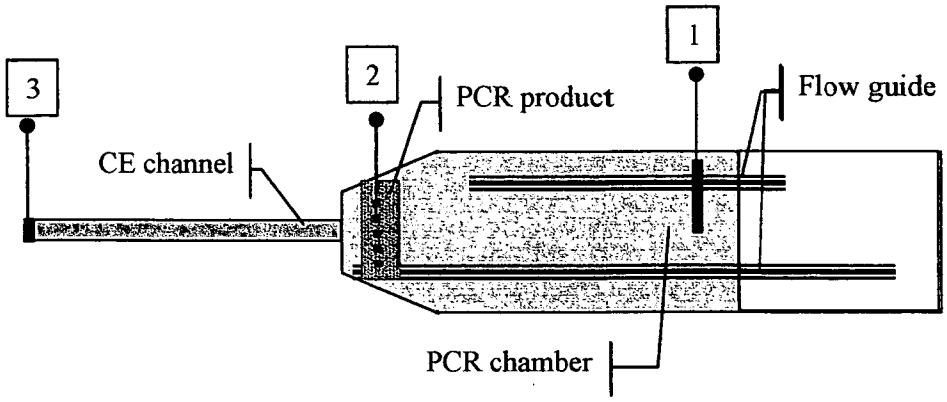
Top view

Fig. 13 . Real time PCR and sequencing detection systems



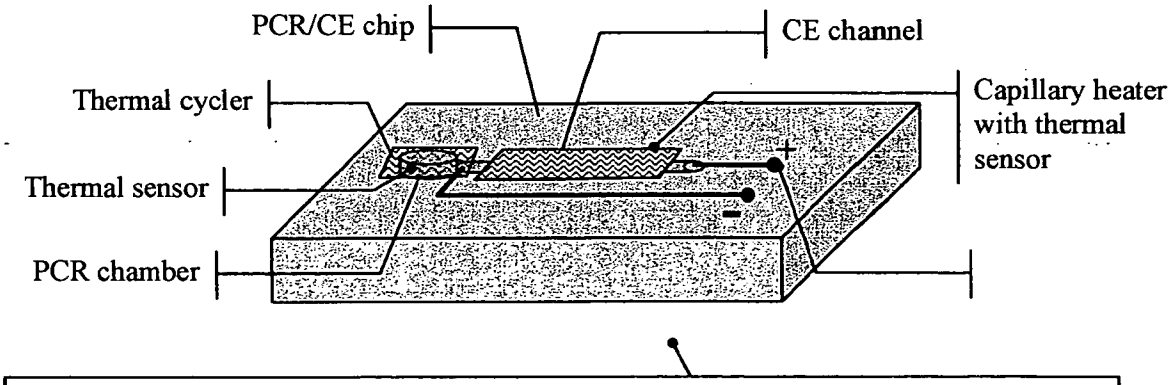
There is a variety of optical systems and photodetectors which can be used for detection of the PCR product and CE separation on the preferred PCE/CE chip. Optical system can have separate illumination for individual PCR chambers and CE channels as well as one or several light sources (lasers, LEDs) which illuminate several chambers (or CE channels) simultaneously.
Design of the optical detection system can be based either on open optics or on fiber optics or comprise both open optics and fiber optics features.
Detection of different channels (and different chambers) can be done in a multiplex mode or simultaneously. In case when detection is carried out simultaneously one can use a multi-pixel photodetectors or single pixel detector. In case of the single pixel detector, illumination of individual PCR chambers and/or individual CE channels must be done by multiple light sources with encoded output power.

Fig.14.Placement of CE electrodes on PCR/CE chip



There is a variety of electrode placements which can be used on the PCR/CE chip. In the preferred embodiment electrodes 1 and 2 are formed inside of the PCR chamber. Applying positive potential to the electrode 2 relative the electrode 1 we can concentrate the PCR product near the inlet of the CE channel. Electrode 3 is in the CE channel. Applying positive potential to the electrode 3 relative the electrode 2 and keeping electrodes 1 and 2 under equal potential we can inject the concentrated PCR product into the CE channel and carry out electrophoretic separation of the PCR product.

Fig.15. Thermal cycling means and thermal sensors for PCR/CE chip



In order to carry out PCR and CE procedures the PCE/CE chip has means for thermal cycling the PCR chamber and applying high voltage to the CE channel.

Fig. 16a. PCR mix and sample loading station

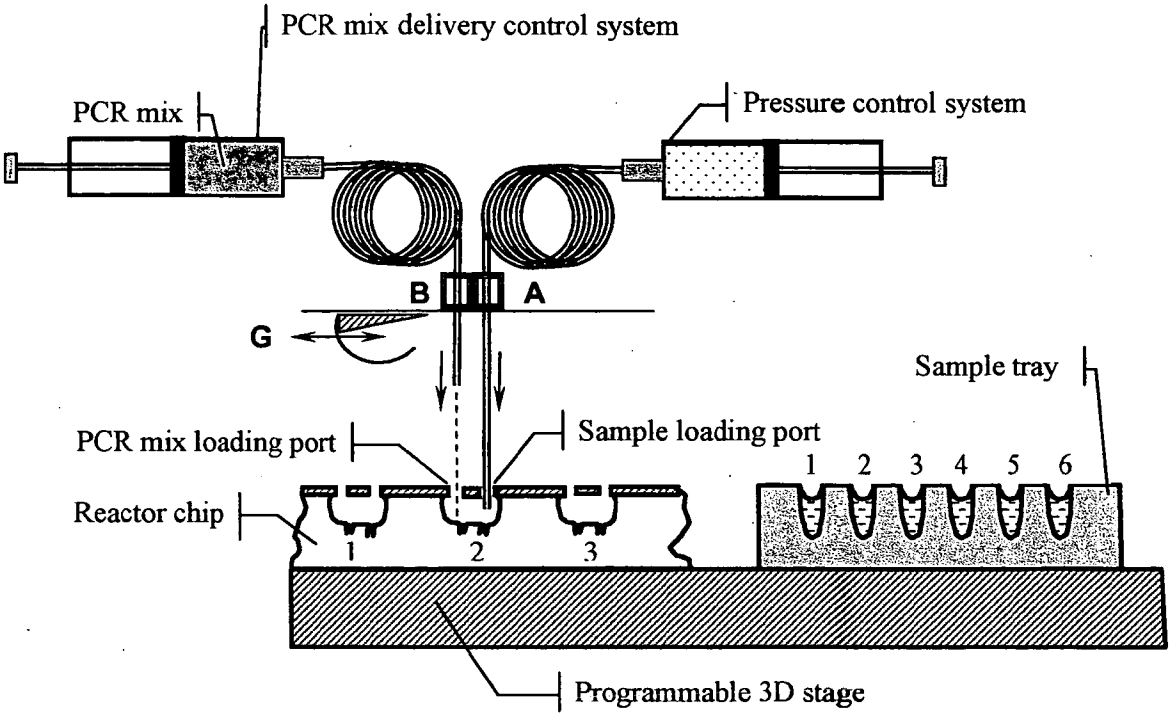


Fig. 16.b. Flexible capillary tube in hydrophobic coating used for loading (and unloading) liquid components.